PB86-118171

Drinking Water Criteria Document for Giardia (Final Draft)

Life Systems, Inc., Cleveland, OH

Prepared for

Environmental Protection Agency, Washington, DC

29 Feb 84

U.S. Department of Commerce
National Technical Information Service
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GIARDIA

PB86-118171

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TR-540-65

FINAL DRAFT FOR THE DRINKING WATER CRITERIA DOCUMENT ON GIARDIA

GIARDIA

February 29, 1984

Prepared Under

Contract 68-01-6750

Program No. 1302

by

ICAIR
Life Systems, Inc.
Cleveland, OH 44122

for

Criteria and Standards Division
Office of Drinking Water
U.S. Environmental Protection Agency
Washington, DC 20460

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INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

2.	3. RECIPIENT'S ACCESSION NO. PB86 118171 /AS
SUBTITLE Drinking Water Criteria Document for Giardia	5. REPORT DATE Feb. 29, 1984
	6. PERFORMING ORGANIZATION CODE
HOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
PERFORMING ORGANIZATION NAME AND ADDRESS Same as box 12.	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection Agency Office of Drinking Water (WH-550) 401 M St., S.W., Washington, D.C. 20460	13. TYPE OF REPORT AND PERIOD COVERED Scientific review
	14. SPONSORING AGENCY CODE EPA/CSD

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The Office of Drinking Water, U.S. Environmental Protection Agency, has prepared a drinking water criteria document on the following topics: general information and properties, occurrence, health effects in animals and humans, mechanisms of disease and risk assessment of Giardia.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Giardia giardiasis Drinking Water		

18. DISTRIBUTION STATEMENT OPEN	19. SECURITY CLASS *(This Report)* Non-sensitive	21. NO. OF PAGES 98
	20. SECURITY CLASS *(This page)* Non-sensitive	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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I. SUMMARY

Giardia is the most commonly identified pathogen causing waterborne outbreaks of gastrointestinal disease in the United States (U.S.). However, no federal or state criteria exist for the assessment or treatment of drinking water containing Giardia. Water treatment methods involving only disinfection (chlorine or ultraviolet irradiation) may be inadequate.

Members of the genus Giardia are flagellated, binucleate protozoans which parasitize the intestinal tract of virtually every class of vertebrates. These protozoans exist in a trophozoite (vegetative) and a cyst (dormant, resistant) form. Trophozoites are pyriform and have two morphologically indistinguishable nuclei, microtubular median bodies and a ventral adhesive disk. They lack mitochondria, smooth endoplasmic reticulum, the Golgi apparatus and lysosomes. Biochemically, the trophozoites are aerotolerant anaerobes which metabolize glucose as their only carbohydrate source.

Because no intermediate hosts are required, the life cycle of Giardia is considered to be simple and direct. Trophozoites attach to the brush border of the small intestine by their ventral adhesive disk. Reproduction of trophozoites is by binary fission. Encystment begins in the small intestine when the trophozoite detaches from the epithelium, becomes round in shape and elaborates a cyst wall. The resultant cyst is excreted with the feces. It is as cysts that Giardia survive passage outside of the host and it is by ingestion of cysts that a new host acquires the infection. Passage of the cyst through the acidic conditions of the stomach appears to initiate excystation (cyst-to-trophozoite transformation) which concludes in the small intestine with the emergence of a pair of binucleate trophozoites. While the process of excystation has been induced *in vitro*, the conditions which induce *in vitro* encystment remain unknown.

There is little agreement regarding the characteristics which should be used to define species in the genus *Giardia*. Characteristics that have been used include host specificity, morphometrics and median body morphology. In 1952, Filice challenged the concept of using *Giardia* body dimensions and host specificity as species criteria. He proposed recognizing three species, *G. duodenalis*, *G. muris* and *G. agilis*, based solely on the morphology of their median bodies. This classification scheme has not ended the debate. Arguments in the recent literature support *Giardia* measurements and host specificity as species criteria. Since no general agreement exists for *Giardia* species nomenclature, the term *Giardia* used in this document refers to organisms of the *G. duodenalis* type unless otherwise indicated. *Giardia duodenalis* includes the human species currently called *G. lamblia*.

Experimental animal infection studies have shown that many *Giardia* species are not highly host specific. *Giardia* cysts from humans have been shown to infect rats, mice, dogs, cats, beavers and gerbils among others. Another study suggested that *Giardia* cysts from beavers and mule deer may infect humans.

Human and animal infections with Giardia (giardiasis) occur endemically worldwide. In humans, infections have been reported from the arctic to the tropics. The Centers for Disease Control (CDC) stated that Giardia is the most commonly reported pathogenic intestinal parasite in the U.S.

The transmission of giardiasis is by the fecal-oral route. The usual method of acquiring the infection is by relatively direct transfer of Giardia cysts to the mouth via food or drink or by hand contact. The study of a number of recent cases of giardiasis, the acquisition of which could not be explained readily by traditional concepts, led to the realization that Giardia infection can be transmitted during sexual contact (especially among homosexual males) and on a larger scale by drinking insufficiently treated surface water. Humans and other animals may serve as sources of Giardia cysts in waterborne outbreaks.

There are epidemiological data to suggest that water was the vehicle of cyst spread in at least 50 outbreaks of giardiasis, infecting approximately 20,000 people in the U.S. from 1970 to 1982. Outbreaks of giardiasis have been reported mainly in New England, the Pacific Northwest and Colorado, but this is undoubtedly due to active surveillance in these areas compared to the rest of the U.S. The waterborne disease surveillance system is a passive system for the most part. States such as Washington, Vermont and Colorado have received federal funds for support of their outbreak surveillance. During 1980 and 1981 when Colorado received federal funds for surveillance, the number of reported outbreaks averaged 7.0 per year as compared to 1.9 per year during 1971 through 1979 when federal funds were not available. Evidence exists for direct person to person transmission of Giardia cysts in day-care centers.

Once thought to be a harmless commensal organism, Giardia is now considered to be pathogenic in both humans and other animals. Most cases of giardiasis appear to be asymptomatic, but symptomatic cases are common and may be acute or chronic. Symptomatic cases have the following traits: diarrhea, abdominal cramps, flatulence, weight loss and steatorrhea. Severe cases may result in malabsorption, growth retardation or celiac-like syndrome, but rarely death.

The human immune response to giardiasis, although not clearly understood, appears to be both humoral and cellular. Reports of successful immunofluorescence tests and enzyme-linked immunosorbent assays hold promise for the development of useful commercially available diagnostic tests, but presently such tests are not available.

A positive correlation has been found between the severity of diarrhea and the severity of villus atrophy in giardiasis, and data from in vitro enzyme studies suggest that sonicates of Giardia trophozoites may inhibit lipolysis. There is no evidence of toxin production by Giardia.

Experiments using human volunteers and human-source Giardia cysts have shown that as few as ten cysts are required to produce human infection. Approximately 56 million people in the U.S. are estimated to be at increased risk from giardiasis via community drinking water systems. Individuals and populations considered to be at greatest risk from giardiasis are: (1) those who drink surface water that is treated by disinfection but not filtration, (2) those who drink untreated surface water, (3) children between the ages of one and three in day-care centers and their families, (4) individuals (especially homosexual males) who engage in sexual activities leading to fecal-oral contact and (5) foreign travelers.

At present, no maximum contaminant level has been established for _Giardia_ in water. A method for analyzing water for _Giardia_ is available. The method, considered tentative and experimental, involves filtering 380 L of water through a microporous filter. After the sediment is removed from the filter, it is concentrated and examined microscopically. If cysts are found, aliquots of the sediment are fed to specific-pathogen-free animals to determine cyst infectivity. This analytical method is currently too unreliable to suggest its use for routine monitoring. The possibility exists that a newly developed immunofluorescence assay could be modified for the detection of _Giardia_ cysts in concentrated water samples.

II. GENERAL INFORMATION AND PROPERTIES

A. Extent and Significance of the Problem

Giardia, an intestinal protozoan parasite with worldwide distribution, is the most commonly reported intestinal parasite in humans in the U.S. (USPHS 1979a). Furthermore, Giardia is the most commonly identified pathogen causing waterborne outbreaks of gastrointestinal disease in this country (USPHS 1981b, 1982b, 1982c). Between 1970 and 1982, at least 50 waterborne outbreaks resulting in approximately 20,000 cases of giardiasis were reported in the U.S. (Craun 1979, USPHS 1981, 1982a, 1982b). Approximately 56 million people in the U.S. are potentially exposed to Giardia infection via community drinking water systems (See Section VII). Despite this, no federal or state criteria or standards exist for the assessment or treatment of drinking water containing Giardia cysts.

Water treatment using chlorine (Jarroll et al. 1981a, Rice et al. 1982) has been shown to inactivate Giardia cysts from human and animal sources. However, the chlorine residual required was at least ten times greater than the 0.2 mg/L residual recommended in the Safe Drinking Water Act of 1974 for bacterial contaminants.

Rice and Hoff (1981) reported that Giardia cysts from human sources were extremely resistant to high doses of ultraviolet irradiation. In their experiments, less than a one log reduction in cyst survival was obtained even when cysts in water were exposed to a maximum dose of 63,000 $\mu W\text{-}s/cm^2$ of ultraviolet irradiation at 254 nm wavelength. They pointed out that this level was far in excess of the minimum dosage of 16,000 μW-s/cm^2 recommended by the U.S. Public Health Service for ultraviolet disinfection of water.

Logsdon et al. (1981) have shown that diatomaceous earth filters with a 1.0 kg/m^2 diatomite precoat should remove a high percentage of *Giardia* cysts. This report was based on studies employing 9-μm-diameter radioactive microspheres and *G. muris* cysts as models.

B. Species of Interest

1. Physical Description of Organism

Members of the genus *Giardia* are flagellated protozoan parasites which belong to the class Zoomastigophorasida, order Diplomonadorida and family Hexamitidae. All of the organisms in this genus are parasites which occur in trophozoite and cyst forms (Levine 1979).

a. Trophozoite

Trophozoites of the genus *Giardia* are the vegetative form of the organism which inhabit the upper small intestine of the vertebrate host (Filice 1952). Their pyriform bodies are from 9 to 21 μm long, 5 to 15 μm wide and from 2 to 4 μm thick (Levine 1979). The trophozoites are identified by the presence of two morphologically indistinguishable anterior nuclei, eight flagella, two central axonemes, microtubular median bodies and a ventral adhesive disk

(Filice 1952, Levine 1979). Trophozoites appear to lack mitochondria, smooth endoplasmic reticulum, the Golgi complex, lysosomes and axostyles (Friend 1966).

Biochemically, *Giardia* trophozoites are aerotolerant anaerobes (Lindmark 1980, Jarroll et al. 1981b). Jarroll et al. (1981b) demonstrated the presence of Embden-Meyerhof-Parnas and pentose phosphate pathways. Additionally, *Giardia* trophozoites are not capable of *de novo* lipid or pyrimidine synthesis (Jarroll et al. 1981b, Lindmark and Jarroll 1982).

The production of disease symptoms in giardiasis has been associated with the attachment of trophozoites to the brush border of the gut epithelium (Veghelyi 1939, Hoskins et al. 1967, Erlandsen and Chase 1974). The mechanism of attachment is not completely understood. Friend (1966) suggested that attachment was accomplished, not by the ventral disk, but by a ventrolateral flange. Holberton (1973, 1974) proposed a hydrodynamic model involving flagellar activity below the ventral disk resulting in negative pressure. Recently, Erlandsen et al. (1979) and Feely et al. (1982) demonstrated the presence of contractile proteins in the ventral disk. They postulated that the presence of actin, α-actinin, myosin and tropomyosin in the disk play an important role in attachment.

Filice (1952) stated that a pair of staining structures that lie dorsal to the axonemes (and are tipped dorsoventrally and anterioposteriorly so that the right tip is more dorsal and anterior) has been found in every species of *Giardia* described. Filice named these structures median bodies. They consist of random arrangements of microtubules that lack an origin or insertion into any other structure (Friend 1966). Friend (1966) suggested that these bodies might play a supporting function in the posterior portion of the trophozoite behind the striated (ventral) disk.

Filice (1952) stated that median bodies are rarely (if ever) seen in cysts. However, Sheffield and Bjorvatn (1977) found a group of randomly arranged microtubules near the flagellar axonemes in cysts and suggested that these may be median bodies. Sheffield and Bjorvatn observed that these microtubules were less compact than those observed by Friend (1966) in the trophozoite, which could account for the apparent absence of median bodies in cysts when viewed with a light microscope.

b. Cyst

Giardia cysts are typically ovoid, measuring from 10 to 15 μm in length and from 7 to 10 μm in width. The cyst wall is approximately 0.3 μm thick (Sheffield and Bjorvatn 1977). Newly formed cysts contain two morphologically indistinguishable nuclei. Each nucleus in the cyst undergoes a single further division so that mature cysts contain four nuclei (Figure II-1). Cysts appear to provide protection from hypotonic lysis, since trophozoites which are shed without cyst walls disintegrate (Figure II-1). Unpublished data from Dr. E. A. Meyer's laboratory suggest that laboratory cultured Giardia lamblia trophozoites enlarge and undergo lysis within 30 minutes after being removed from culture medium and placed in tap water. Although these data have not been published, they are consistent with the fact that no osmoregulatory

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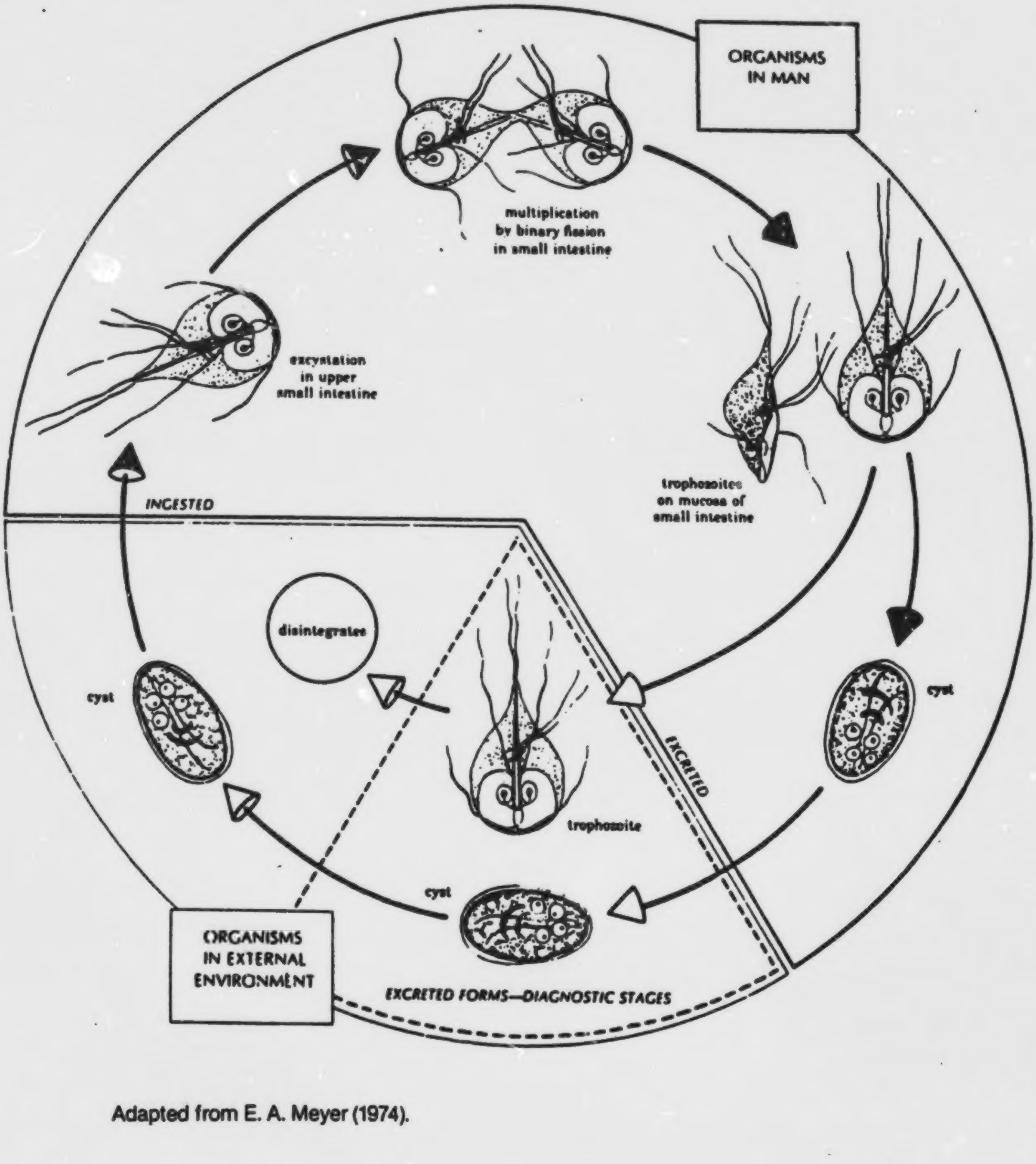
ORGANISMS IN MAN
multiplication by binary fission in small intestine
excystation in upper small intestine
trophozoites on mucosa of small intestine
INGESTED
disintegrates
cyst
trophozoite
EXCRETED
cyst
cyst
ORGANISMS IN EXTERNAL ENVIRONMENT
EXCRETED FORMS—DIAGNOSTIC STAGES

Adapted from E. A. Meyer (1974).

FIGURE II-1 LIFE CYCLE OF GIARDIA

systems have been reported in these or other cyst-forming parasitic protozoa. Encysted forms remain capable of excystation for up to 2 months, even when stored in water (Bingham et al. 1979).

2. Life Cycle of Giardia

Giardia have a life cycle in which the flagellated, binucleate trophozoites reside in the upper two thirds of the host's small intestine (the duodenum and jejunum), where they attach, possibly by means of a ventral adhesive disk, to the brush border of the epithelium and reproduce by binary fission (Figure II-1). The trophozoite-to-cyst transformation takes place in the intestinal tract (Filice 1952) (Figure II-1). The cycle proceeds following excretion of the cyst and its subsequent ingestion by a suitable vertebrate host (Hegner 1922, Filice 1952, Rendtorff 1954, Grant and Woo 1978b).

Sometimes the transition in the intestine from trophozoite to cyst fails to occur. This may happen in instances of rapid intestinal transit when residence time is insufficient for cyst formation. Diarrheic stools from patients with giardiasis thus are particularly likely to contain trophozoites (Ormiston et al. 1942, Moore et al. 1969).

a. *In vivo* Excystation

The passage of viable cysts through the stomach appears to initiate the process of excystation, which is completed in the small intestine (Filice 1952). At the time of excystation, a quadrinucleate trophozoite in the process of division emerges from the cyst wall and promptly completes the division process, yielding two binucleate trophozoites (Bingham and Meyer 1979).

b. *In vitro* Excystation

Bingham and Meyer (1979) successfully induced *in vitro* excystation of *Giardia* cysts from human feces. The cysts were separated from the feces by a combination of filtration and purification employing centrifugation and layering of cysts on 0.85 M sucrose (Bingham et al. 1979).

Bingham and Meyer (1979) found that exposure of purified cysts to synthetic gastric juice at pH 1.6 induced excystation within 30 minutes. When the individual components of the gastric juices were tested, only HCl was required to induce excystation. The exposure of *Giardia* cysts to a variety of acids at pH 2.0, regardless of the counterion, induced significantly higher levels of excystation than the water controls. The authors concluded that the hydrogen ion rather than the counterion was necessary to induce excystation of *Giardia*.

Two recent techniques have appeared that seem to improve both the cyst purification and excystation methods of Bingham and Meyer (1979). Sauch (1983) has reported, in an abstract, that a Percoll solution at pH 7 and 1.08 g/cm^3 gave consistently higher cyst recoveries than either the sucrose or zinc sulfate methods. In order to improve the purification of the recovered cysts, the cyst suspensions were centrifuged at different sedimentation velocities at unit gravity in Percoll density gradients from 1.01 to 1.03 g/cm^3. No mention was made of the effect of Percoll, if any, on cyst viability.

Rice and Schaefer (1981) reported consistently higher levels of Giardia excystation than those obtained by Bingham and Meyer (1979). The Rice and Schaefer procedure requires the use of a low-pH induction step, involving three separate solutions, and an excystation step. In the inducement protocol, aqueous HCl (pH 2.0), 1x Hanks balanced salt solution containing cysteine HCl and glutathione, and sodium bicarbonate (0.1 M) were added to a cyst suspension. This mixture was incubated for 30 minutes at 37° C. Following the incubation, cysts were removed from the inducement medium by centrifugation and excystation medium was added.

In the excystation step of the Rice and Schafer procedure, the excystation medium contained trypsin (0.5% W/V) and 1x Tyrode solution and was adjusted to pH 8 with sodium bicarbonate. After an hour of incubation, the solution was centrifuged and cysts were resuspended in 0.5 mL of fresh excystation medium. Slide chamber preparations and counting procedures used were the same as those described by Bingham et al. (1979). The levels of excystation in these experiments ranged from 40 to 95%.

c. Encystment

During the intestinal portion of the Giardia life cycle, some of the trophozoites detach from the brush border for unknown reasons and enter the fecal stream. Encystment begins in the small intestine (Filice 1952) with the trophozoites becoming rounded and elaborating a cyst wall; the resultant cysts are excreted with the feces (Figure II-1). The conditions required to trigger the encystment process *in vitro* have not yet been determined.

Danciger and Lopez (1975) recorded *Giardia* cyst production in 15 infected children over a 1- to 3-month period. Three patterns of cyst excretion were observed. In some, termed high cyst excreters, large numbers of cysts were present in nearly all stool samples. By contrast, low cyst excreters had detectable levels of cysts in only 40% of their stools. The third group, "mixed excreters", had 1- to 3-week periods of high cyst excretion alternating with periods of low cyst excretion. No correlation was found between the numbers of cysts produced and the consistency of the stool or frequency of defecation. Furthermore, the use of purgatives failed to increase cyst production (Danciger and Lopez 1975).

Grant and Woo (1979) observed cyclic cyst excretion by laboratory rats and mice experimentally infected with *Giardia simoni* and *Giardia muris*, respectively. This cyclic cyst excretion with periods of 7-8 days between peaks was also observed in captive deer mice and meadow voles that were naturally infected with *Giardia*.

Rats infected with *Giardia lamblia* from humans appear to have a similar cyst excretion pattern to that observed in humans by Danciger and Lopez in 1975 (Craft 1982). Craft observed that 12% of the rats excreted large numbers of cysts continuously while 80% excreted variable numbers of cysts intermittently. No cysts were excreted by 8% of the infected rats.

3. Distribution of Giardia Species in Nature

Organisms in the genus Giardia have been reported as intestinal inhabitants of a variety of mammals, birds, reptiles, amphibians and fish and should be considered among the most widely occurring of the intestinal protozoan parasites (Table II-1). Many of these Giardia species cannot easily be distinguished from one another morphologically (Filice 1952).

Early workers assumed that Giardia were highly host specific (Hegner 1922, Travis 1939). It is mostly on this basis that the species listed in Table II-1 were described. More recent work suggests that, while the Giardia in some animals may exhibit an apparent high degree of host specificity, other Giardia species are capable of infecting more than one host species (Armaghan 1937, Davies and Hibler 1979, Grant and Woo 1978b).

4. Species Concepts in the Genus Giardia

There is no general agreement regarding those characteristics which define species in the genus Giardia. Characteristics which have been used previously include host specificity, morphology and variations in the shape of the median bodies (Hegner 1922, Hegner 1925, Hegner 1926c, Travis 1939, Simon 1922, Filice 1952, Grant and Woo 1978a,b).

The suitability of relying on Giardia body dimensions as a means of speciating these parasites has been questioned (Tsuchiya 1931, Filice 1952). While Giardia size and shape are known to vary with organisms from certain

Table II-1 Reported *Giardia* Hosts, 1859-1928

Species Name	Name of Describer	Date	Host
Mammals			
Giardia lamblia.........	Lambl	1859	Man
Giardia pitymisi........	Splendore	1920	Vole
Giardia duodenalis......	Devaine	1875	Rabbit
Giardia muris...........	Grassi	1879	Rat and mouse
Giardia microti.........	Kofoid and Christiansen	1915	Field mouse
Giardia canis...........	Hegner	1922	Dog
Giardia caviae..........	Hegner	1923	Guinea pig
Giardia viscaciae.......	Lavier	1923	Viscacha
Giardia caprae..........	Nieschulz	1923	Rat
Giardia simoni..........	Lavier	1924	Goat
Giardia equi............	Fantham	1921	Horse
Giardia bovis...........	Fantham	1921	Cow
Giardia sp..............	Hegner	1924	Wild cat
Giardia sp..............	Deschiens	1925	Lion
Giardia cati............	Deschiens	1925	Cat
Giardia sp..............	Hegner	1924	Monkey
Giardia sp..............	Nieschulz	1923	Calf
Giardia suricatae.......	Fantham	1923	Meercat
Giardia beckeri.........	Hegner	1926	Ground squirrel
Giardia otomysis........	Fantham	1926	*Otomys irroratus*
Giardia bradypi.........	Hegner and Schumaker	1928	Three-toed sloth
Birds			
Giardia sanguinis.......	Gonder	1910	*Elanus caeruleus* (blood)
Giardia ardeae..........	Noller	1920	Heron
Giardia sp..............	Kotlan	1922	*Lanius collurio*
Giardia sp..............	Kotlan	1922	*Recurvirostra avacetta*
Giardia sp..............	Hegner	1925	Black-crowned night heron
Giardia sp..............	Hegner	1925	Great blue heron
Reptile			
Giardia varani..........	Lavier	1923	*Varanus niloticus*
Amphibians			
Giardia agilus..........	Kunstler	1882	Tadpole
Giardia xenopi..........	Fantham	1923	Clawed frog
Fish			
Giardia denticis........	Fantham	1919	Fish (blood)
Nematode			
Giardia sp..............	Thomson	1925	*Viannella* sp.

Adapted from Hegner and Andrews (1930).

hosts, it is also true that Giardia isolated from a number of different hosts may be morphologically indistinguishable (Filice 1952, Grant and Woo 1978a).

a. Filice's Concept

Filice (1952) addressed the question of Giardia speciation by measuring, then statistically comparing, the body dimensions of Giardia trophozoites from different animal species. He concluded that the use of differences in body dimensions of these organisms and host specificity were untrustworthy species criteria. Filice (1952) then suggested a conservative approach to the problem: he proposed basing Giardia species designations primarily on structural differences. He recognized, however, that physiologically distinct species may exist among those that appear similar morphologically. The organelle which occurs in Giardia and which Filice (1952) proposed using as the basis for speciation was the median body. Filice proposed recognizing the following Giardia species:

- G. duodenalis, whose single or double median body somewhat resembles the claw of a claw hammer. These organisms have been isolated from humans, other mammals (including rodents), birds and reptiles.

- G. muris, with two small rounded median bodies in the center of the organism. Rodents, birds, and reptiles have been shown to be hosts for this morphological type of Giardia.

- *G. agilis*, with long, teardrop-shaped median bodies. While the adhesive disk of the other two proposed species is on the order of half the trophozoite body length, the *G. agilis* adhesive disk is approximately one-fifth the body length of these organisms. Organisms of the *G. agilis*-type have only been described from amphibian hosts.

Filice (1952) suggested assigning nontaxonomic status, such as race, to his three proposed species so as to incorporate *Giardia* presently considered species on the basis of size or host specificity alone.

b. Other Concepts

Grant and Woo (1978a, 1978b) have recently attacked the problem of speciation from two directions, namely cross-species transmission studies and morphometrics. Their work involved *Giardia* from small mammals in Ontario, Canada, and they were able to distinguish five species of *Giardia*: *G. muris*, *G. mesocricetus*, *G. simoni*, *G. microti* and *G. peromysci*. These five species were divided into two types.

In type I, trophozoites have elongated nuclei in the posterior region of the sucking disk, the sucking disk occupies a large portion of the body, basal bodies are anterior to the nuclei, and the median bodies are round or oval and near the center of the body. This type includes *G. muris* and *G. mesocricetus*.

In type II, trophozoites are reported to be longer than they are wide, the sucking disk is in the anterior half of the body, the nuclei are in the central region of the disk and the median bodies are comma or claw-hammer shaped. Type II trophozoites include *G. simoni*, *G. microti* and *G. peromysci*.

Grant and Woo (1978a) were not able to distinguish any further characterization of these species within each type based on morphological observations, but subsequently noted differences among the major dimensions of some of the trophozoites within and between the two types.

The type I trophozoites, *G. muris* and *G. mesocricetus*, were statistically different from each other (and in general from type II trophozoites) in body length and width. Among type II trophozoites, *G. simoni* was significantly different from G. *peromysci* in all body dimensions and from *G. microti* in all dimensions except length. *Giardia microti* and *G. peromysci* differed only in body length.

Grant and Woo (1978a) followed the method of Solov'ev (1975) and plotted the ratios of average body length to the average body width with standard errors. Even though there was considerable overlap among the various species, this plot revealed significant differences ($p>0.05$) among *G. microti*, *G. simoni*, *G. muris* and *G. peromysci*.

Grant and Woo (1978a) discussed a variety of problems dealing with *Giardia* speciation based on morphometrics. They reported differences in size, general morphology, density of cytoplasmic staining and relative proportions of cytoplasmic organelles of *Giardia* trophozoites under the influences of prefixation drying times. The only morphological characteristic which remained unaltered by prefixation treatment was the median body. These authors suggested that in addition to the median body, overall body dimensions are useful if consistent methods of fixation and staining are used.

The absence of median bodies in some of the trophozoites examined by Grant and Woo (1978a) led them to conclude that trophozoites in the same animals may be in different stages of development. Since trophozoites lacking median bodies were consistently smaller than those with median bodies, they used only trophozoites with median bodies present.

The second thrust of the Grant and Woo (1978b) study was the establishment of *Giardia*-free mouse and rat colonies (discussed in detail in Section II.B.5.) to examine host specificity of the two types of *Giardia* they discerned morphologically. They concluded that *G. simoni*, *G. muris* and *G. peromysci* were host specific but that *G. microti* and *G. mesocricetus* were not.

From these two studies, Grant and Woo (1978b) concluded that species of *Giardia* should be defined using a combination of morphological, morphometric and host-specificity criteria. No such comprehensive studies have been conducted for *Giardia* isolated from humans.

5. Cross-Species Transmission Studies

a. Transmission to and from Humans

Simon (1922) performed cross-transmission studies in which laboratory rats and wild rats were fed Giardia cysts washed from human feces. His attempts at cross-transmission were unsuccessful. It should be noted here and in much of the early work on cross-species transmission that the viability of the source cyst was either unknown or assessed by eosin staining and, thus, failure of transmission could be due to feeding nonviable cysts rather than species specificity.

Bonestell (1935) reported G. lamblia trophozoites in the wood rat intestine after they ingested the cysts. Sehgal et al. (1976) reported similar results in adult and weanling albino rats with Giardia cysts from humans. In the latter study, the infection rate was low but it increased once the parasite had passaged through rats ($p<0.05$). Furthermore, Sehgal et al. (1976) noted that more weanling rats were infected than adult rats, but the difference was not significant ($p>0.05$). Vinayak et al. (1979) reported 100% infection of 17 weanling Swiss mice (3-4 weeks old, 17-22 g) given a minimum of 150,000 G. lamblia cysts.

Davies and Hibler (1979) obtained Giardia cysts from humans and beavers and attempted to infect a variety of wild and domestic animals. In their studies with Giardia cysts from humans, varied results were reported. Attempts to infect hamsters, domestic rabbits, laboratory mice, deer mice, cattle, wapiti, mule deer, white-tailed deer, black bear and domestic sheep were unsuccessful. Success was reported in attempts to infect laboratory rats, gerbils, guinea pigs, beavers, dogs, raccoons, bighorn sheep and pronghorns (Table II-2).

Two out of three human volunteers became cyst-positive after ingestion of Giardia cysts from a beaver, one within 6 days and the other at 11 days (Davies and Hibler 1979). When Giardia cysts collected from beavers were fed to laboratory mice, rats, guinea pigs and hamsters, none of these animals became infected (Davies and Hibler 1979). However, when cysts from the same source were given to four beagle puppies, all were cyst-positive within 8 days. The control animal was positive 9 days after cysts were observed in the four experimental pups. It is not clear whether the infected control animal was housed with the four challenged animals. Davies and Hibler (1979) reported that Giardia cysts from a naturally infected mule deer failed to infect two beagle puppies but one human volunteer began shedding cysts 9 days after exposure (Tables II-3 and II-4). It appears that these human infection studies were performed under less than optimally controlled conditions, and that the carrier status of the human volunteers prior to ingestion of the cysts was not well established. Such verification would seem mandatory in light of the prevalence of giardiasis in Colorado. These volunteers no doubt had other potential sources of exposure to Giardia.

Hewlett et al. (1982) used Giardia cysts from asymptomatic and symptomatic human donors and axenically cultured trophozoites originally

Table II-2 Results of Experimental Infections Using Human-Source *Giardia*

Experimental Animal	Exposure[a]	Results
Hamster (*Mesocricetus auratus*)	4+1	Negative - 34 days.
Domestic rabbit (*Oryctolagus cuniculus*)	4+1	Negative - 28 days.
Laboratory mouse (*Mus musculus*)	4+1 (4 groups)	All negative - 40 dyas.
Deer mouse (*Peromyscus maniculatus*)	4+1 (2 groups)	Negative - 22 days.
Rat (*Rattus norvegicus*)	4+1	Composite fecals - positive - day 22, 25 and 40 (cysts of 2 sizes).
	4+1	One rat positive 34 days post-exposure for 1 day.
Gerbil (*Gerbillus gerbillus*)	4+1	No. 1 Positive day 22, 26-29, 32-33. No. 2 Positive day 13, 19-22, 26-28. No. 3 Control-positive day 33-35. No. 4 Positive day 18-21, 26. No. 5 Negative 45 days.
	4+1 (2 groups)	One gerbil was positive for 1 day at 8 days post-exposure.
	4+1	Negative - 42 days.
Guinea pig (*Cavis porcellus*)	4+1	One animal became positive on day 21 and remained positive for 31 days. All others were negative.
Beaver (*Castor canadensis*)	2+1	Negative - 38 days.
	2+1	Both positive 25 days after exposure cysts seen for 22 days.
Dog (*Canis familiaris*)	2+1	Positive 6 days post-exposure for 14 days then animals were treated.
	2+1	Positive 8 days post-exposure for 14 days then animals were treated.

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[a] Number of exposed animals + number of in-group controls.

continued-

Table II-2 - continued

Experimental Animal	Exposure[a]	Results
Raccoon (Procyon lotor)	3+1	Negative - 28 days.
	3+1	Negative - 43 days.
	3+1	Negative - 34 days.
	1	Positive 8 days post-exposure for 1 day - Negative for the next 20.
Black bear (Ursus americanus)	1	Negative - 28 days.
Bighorn X mouflon sheep (Ovis canadensis X O. musimon)	4+1	No. 1 Negative. No. 2 Negative. No. 12 - Control - Negative. No. 29 - Positive days 9-13 post-exposure. No. 44 - Positive days 9-13 post-exposure.
Wapiti (Cervus canadensis)	2+1	Negative - 30 days.
	2+1	Negative - 28 days.
Mule deer (Odocoileus hemionus)	2+2	Negative - 28 days.
	4+2	Negative - 30 days.
	3+6	Negative - 22 days.
Pronghorn (Antilocapra americana)	1+1	Positive days 16-18 post-exposure.
Domestic sheep (Ovis aries)	3+1	Negative - 45 days.
Domestic cattle (Bos tarus)	4+1	Negative - 45 days.
White-tailed deer (Odocoileus virginianus)	1+2	Negative - 30 days.
	1+6	Negative - 22 days.

Adapted from Davies and Hibler (1979).

Table II-3 Results of Experimental Infections Using Beaver (Castor canadensis)-Source Giardia

Experimental Subject	Exposure[a]	Results
Mouse (Mus musculus)	4+1 (2 groups)	All negative.
Rat (Rattus norvegicus)	4+1 (2 groups)	All negative.
Hamster (Mesocricetus auratus)	4+1	Negative - 30 days.
Guinea pig (Cavia porcellus)	4+1	Negative - 30 days.
Dog (Canis familiaris)	4+1	All 4 exposed animals positive 8 days after exposure - control positive 9 days later - all positive 21 days post-exposure.
Human (Homo sapiens)	3+1	No. 1 Positive 11 days post-exposure. No. 2 Positive 6 days post-exposure. No. 3 Negative - subject was on Tetracycline therapy.

[a] Number of exposed subjects followed by number of in-group controls.

Adapted from Davies and Hibler (1979).

Table II-4 Results of Experimental Infections Using Mule Deer (*Odocoileus hemionus*)-Source *Giardia*

Experimental Subject	Exposure[a]	Results
Dog (*Canis familiaris*)	2+5	Negative 28 days.
Human (*Homo sapiens*)	1	Positive 9 days post-exposure.

[a] Number of exposed subjects followed by number of in-group controls.

Adapted from Davies and Hibler (1979).

isolated from a human to assess cross-species transmission of Giardia to mongrel dogs. The dogs were obtained from an animal shelter and screened by stool examination for pre-existing Giardia infection. Of the eight dogs that were given from 5×10^5 to 3.2×10^6 Giardia cysts orally, three became infected. One of those three dogs that shed cysts for 17 days, one for 42 days and one for longer than 166 days. The dog that excreted cysts for 17 days shed a maximum of 3×10^3 cysts/g of feces while the dogs that excreted for 42 days and greater than 166 days shed a maximum of 1.9×10^5 and 1.06×10^6 cysts/g of feces, respectively. Two of the three dogs inoculated orally with 1.5×10^6 Giardia trophozoites in culture medium became infected and shed from 8×10^4 to 1.3×10^5 cysts/g of feces.

From these studies, Hewlett et al. (1982) concluded that (1) infection with G. lamblia is not restricted to humans, (2) the trophozoite form can be infectious and (3) dogs, particularly household pets, should be considered as a possible source of infection.

Another approach to examining the cross-species transmission of Giardia was presented by Craft (1982). In this study, pathogen-free Sprague-Dawley rats were screened for Giardia infections by microscopic examination and counterimmunoelectrophoresis of feces (Craft and Nelson 1982) prior to experimentation. Giardia cysts were concentrated as described by Sheffield and Bjorvatn (1977) from a human with symptomatic giardiasis. Rats were given a single dose of cysts by micropipet. By the seventh day after infection virtually all of the rats were positive for Giardia infection as determined by intestinal biopsy and counterimmunoelectrophoresis. Direct fecal examination demonstrated cysts in only 40% to 70% of these rats. Craft (1982) concluded that transmission of Giardia from humans to rats was consistently possible and pointed out that the method of detection of giardiasis in experimental animals was critical. Duplication of this work has not appeared in the literature.

Hill et al. (1983), using a challenge dose of 20,000 trophozoites of axenically cultured Giardia of the G. duodenalis-type (lamblia-type), were able to consistently infect suckling albino CF-1 mice at 3, 7 and 14 days of age. Cysts which were produced and excreted were infective for other mice of the same age. Infection was cleared by all mice within 17 to 21 days. The age of the mouse was apparently critical in determining whether or not infection ensued. This is because no challenged mice 21 days or older became infected, nor did any of the mothers of the suckling infected litter.

Belosevic et al. (1983) have reported that 6- to 10-week-old male Mongolian gerbils (Meriones unguiculatus) were susceptible to infection with G. lamblia. Each animal received 5,000 cysts or 10^6 trophozoites, and cysts were excreted for 30 days.

Perhaps the best-documented studies of feeding Giardia cysts from humans to other humans are those of Rendtorff (1954) and Rendtorff and Holt (1954). Rendtorff demonstrated that Giardia cysts, ingested either in capsules or in water, were capable of becoming established in the challenged host, as determined by the pattern of stool excretion containing Giardia cysts. It is of interest that in Rendtorff's study: (1) most infections disappeared spontaneously, (2) different patterns of infection were observed, depending on the donor source of cysts (suggesting to Rendtorff (1979) that there may be strain differences in this species), and (3) some infected persons failed to shed cysts for a long period. Although Giardia infection was accompanied by a change in the stool pattern, Rendtorff was unable to determine with certainty a relationship between Giardia infection and clinical complaint or subjective illness.

b. Transmission from Animal to Animal

Hegner (1922, 1924, 1926b, 1926c, Hegner and Andrews 1930) was convinced that Giardia are strictly host specific. Despite this, he reported that Giardia cysts from dog feces excysted in the intestine of a guinea pig (Hegner 1926a). Similar observations were noted with Giardia cysts from a human introduced into laboratory rats (Hegner 1927).

Box (1981) isolated Giardia cysts from pooled parakeet feces and administered them by gavage to two canaries and three DBA-2 mice. Except for one canary, these animals received between 60-110 x 10^3 cysts. One of the mice and one of the canaries received cysts that were stored in the refrigerator for 1 to 3 weeks. The other mice received cysts that had been refrigerated for 1 to 3 days. One of the canaries received 250 x 10^3 cysts that had been refrigerated for 1 day. None of these five animals became infected.

Grant and Woo (1978b) criticized earlier workers whose experimental animals were not raised in meticulously maintained Giardia-free colonies.

They further criticized the early experiments of Armaghan (1937), Filice (1952) and Bemrick (1962) because the experimental animals were given antigiardial chemotherapy prior to infection attempts but not all of these treatments were totally effective. Furthermore, Grant and Woo (1978b) believed that these treatments may have adversely affected subsequent establishment of the experimental infections.

Grant and Woo (1978b) reported that they had avoided these complications by establishing *Giardia*-free colonies of mice and maintaining these mice in cages with filter tops and wire bottoms and by autoclaving the food, cages and water bottles. As a result of their experiments they concluded that *G. simoni*, *G. muris* and *G. peromysci* were host-specific while *G. microti* and *G. mesocricetus* were not.

Sebesteny (1979) used specific-pathogen-free mice (unidentified strain) raised by the Imperial Cancer Research Fund and successfully infected them with *Giardia* from hamsters. No attempt was made to identify the species of *Giardia* used in their experiments.

C. Summary

Giardia is the most commonly identified pathogen causing waterborne outbreaks of gastrointestinal disease in the U.S. (at least 50 outbreaks between 1970 and 1982 with approximately 20,000 cases reported). Potentially, 56 million people in the U.S. may be exposed to *Giardia* infection via community drinking water systems. However, no federal or state criteria exist for the assessment or treatment of drinking water containing Giardia. Water treatment methods involving only disinfection (e.g., chlorine or ultraviolet irradiation) may be inadequate.

All of the organisms in the genus Giardia are binucleate, flagellated protozoan parasites of the upper gastrointestinal tract in humans and other vertebrates. The parasites exist in trophozoite and cyst forms.

In the Giardia life cycle, trophozoites, which divide by binary fission, attach to the brush border of the small intestinal epithelium, detach for unknown reasons, then become rounded and elaborate a cyst wall. The dormant, resistant cyst is excreted in the feces and transmission to a new host is accomplished by ingestion of viable cysts. Excystation yields trophozoites which promptly attach to small intestinal epithelium.

Excystation is induced by exposure to low pH acids, and has been induced *in vitro*. The mechanism of encystment is unknown.

Giardia have been reported in a variety of mammals and in lower vertebrates. While numerous species of Giardia have been described, there is no general agreement on those criteria which define species in this genus. Criteria used to date include: host specificity, body size and shape, and the morphology of a microtubular organelle, the median body. This organelle appears to be unique to Giardia trophozoites.

Although many early studies were poorly controlled, more recent studies suggest that cross-species transmission of *Giardia* occurs. Experimental human and animal infection studies offer increasing evidence that rats, dogs, cats, beavers, mice, gerbils and mule deer can harbor human *Giardia* and humans appear to have been infected by *Giardia* of the *duodenalis*-type isolated from beavers and mule deer. Furthermore, it seems that these experimental infections may be established by feeding cysts or cultured trophozoites. Mice and rats, experimentally infected with *G. lamblia*, appear to produce a relatively low number of cysts. The necessity for meticulous attention to the use of *Giardia*-free animals is discussed. For determining *Giardia*-free status in experimental animals, reliance on stool examinations alone is not sufficient.

III. OCCURRENCE

A. Specific Occurrence

1. Data from Travelers

Human infections with Giardia occur worldwide. Evidence that giardiasis is not necessarily acquired in the tropics has come from epidemiologic studies of giardiasis acquired by travelers both inside and outside the U.S. Although the most frequent cause of travelers' diarrhea is conceded to be bacterial, several groups of workers (Babb et al. 1971, Morris et al. 1976) have pointed out that giardiasis is responsible for at least some of the cases of this syndrome.

Reports first appearing in 1970 and continuing to the present describe giardiasis among travelers to the Soviet Union. Walzer et al. (1971) were the first to report an outbreak of epidemic giardiasis among American travelers to the Soviet Union. Of 80 people on a tour of the Soviet Union, 23 experienced symptomatic giardiasis. A later report of giardiasis in travelers to the Soviet Union counted 324 of 1,419 travelers from 1969 to 1973 as positive for symptomatic giardiasis (Brodsky et al. 1974). Jokipii and Jokipii (1974) reported that 32 Finnish students who traveled to the Soviet Union between 1971 and 1973 exhibited Giardia-positive stools upon their return to Finland. Drinking water in the city of Leningrad has been repeatedly implicated as a likely source of infection.

Lopez et al. (1978) described an outbreak of giardiasis among a group of travelers to Madeira Island, Portugal. Although drinking tap water on the island was statistically associated with acquisition of the disease, eating ice cream or raw vegetables on the island was also implicated. Thompson et al. (1974) described an outbreak of giardiasis among a group of children and adults on a Mediterranean cruise. The research of Wright et al. (1977a) shows that travelers to Colorado mountains may acquire giardiasis by consuming untreated drinking water. These workers studied more than 500 residents of Colorado and concluded that the disease was more likely to occur in those who visited the mountains, camped overnight and drank untreated mountain water.

2. Data from Day-Care Centers

Environments where numbers of infants and young children are collectively cared for, particularly where the supervision may be inadequate, are suited to the spread of Giardia. The first definitive study of Giardia infection in such a setting is apparently that of Ormiston et al. (1942) in England. Brown (1947) in England noted that the incidence of Giardia infection in 139 nursery children aged 1 to 3 years screened upon admission was 26.5%. Black et al. (1977) described outbreaks of giardiasis in day-care centers in Atlanta, GA. In three day-care centers surveyed, the infection rate for giardiasis ranged from 29 to 54%, whereas only 2% of the children not attending day-care centers were infected. Keystone et al. (1978) reported infection rates of 17 and 39% in children in day-care facilities in metropolitan Toronto, Canada. The infection was reported to have been spread to as many as 23% of the children's household contacts. In both the Black et al. (1977) and

Keystone et al. (1978) studies, the most susceptible age group was 1 to 3 years.

Harter et al. (1982) surveyed 518 children between 1 and 3 years old and their families in two counties in the state of Washington. The study revealed a Giardia infection rate of 7.1% among the children. In the families of Giardia-positive children, 10% of the 47 family members were also Giardia positive. These investigators found no differences in prevalence between children who normally attended day-care centers and those who did not. No correlation was observed regarding the socioeconomic status of the families and the presence of Giardia. The only risk factors identified in this study were a history of drinking untreated surface or well water, and having two or more siblings between the ages of 3 and 10 years old.

3. Data from Homosexual Populations

Evidence exists for transmission of giardiasis by sexual activity, particularly among male homosexuals. Mildvan et al. (1977) presented two cases of male homosexuals who experienced simultaneous or sequential occurrence of giardiasis, shigellosis and amebiasis. Among three homosexuals studied by Hurwitz and Owen (1978), seven intestinal protozoa including Giardia were detected. Meyers et al. (1977) reported four successive cases of giardiasis which were linked to homosexual activity among the individuals.

Kean et al. (1979) observed that 18.3% of 126 homosexual males in a selected New York City population were cyst-positive for Giardia. By comparison, they noted that only 2.1% of 5,885 patients examined for parasitological diseases by the same laboratory using the same procedures were positive for Giardia.

4. Data From Foodborne Outbreaks

Foodborne giardiasis occurred in Minnesota in 1979 (Osterholm et al. 1981). The outbreak occurred among employees, but not students, of a public school in Goodhue, MN. Twenty-nine of the 60 employees of the Goodhue school experienced diarrhea between November 2 and November 23. Fifteen of the 29 ill employees and 2 of the 31 healthy employees were positive for Giardia cysts.

Although no epidemiological association could be made for a common social event or history of water consumption in this study, subsequent questionnaires revealed two food items (prepared by employees and served in the employees' lounge) were statistically associated with the outbreak. The two items were home-canned salmon and cream cheese dip. Cysts were not demonstrated in unopened jars of salmon.

Further investigation, however, revealed that the wife of an employee had prepared the salmon and had touched the salmon by hand before it was given to the employees at Goodhue. She had reportedly diapered her 12-month-old grandson just prior to preparing the salmon. Although she was reported to be free of Giardia symptoms herself, subsequent examinations of the grandson's stool were positive for Giardia. The grandson lived in Hawaii and occasionally attended day-care facilities there.

5. Data from Waterborne Outbreaks

The number of cases of giardiasis from all sources reported to CDC was 11,013 in 1980 and 12,184 in 1981 (USPHS 1981a, 1982a). In 1980 and 1981 respectively, 1,724 (15.7%) and 297 (2.4%) of these cases were waterborne. The decrease in the number of waterborne outbreaks in 1981 may well have been due to less complete reporting than an actual decrease (USPHS 1982c). The waterborne disease surveillance system is, for the most part, a passive one. Supporting this is the fact that four states reported 59% of all 1981 outbreaks (USPHS 1982c). Three of these states, Colorado, Vermont and Washington received federal funds for surveillance in 1981, through Environmental Protection Agency (EPA) contracts, and the fourth, Pennsylvania, has an extremely well-developed surveillance system (USPHS 1982c). Colorado received these federal funds for surveillance in both 1980 and 1981, and during those years reported an average of 7 outbreaks per year. This is in contrast to its previous average of 1.9 outbreaks per year from 1971 through 1979 (USPHS 1982c).

Giardia has been the most commonly identified pathogen causing waterborne outbreaks of gastrointestinal disease in the U.S. since 1965 (Craun 1979, USPHS 1981b, 1982b, 1982c). Waterborne outbreaks were not reported prior to 1965 most likely because of the early belief that *Giardia* did not cause disease.

In retrospect, the earliest noted, and perhaps the largest, community-wide outbreak of an intestinal disease presumed to be giardiasis occurred in

Portland, OR, in 1954-55 (Veazie et al. 1979). Fifty thousand cases of intestinal disease were calculated to have occurred, and the only frequently observed organism from these cases was *G. lamblia*. Nevertheless, because at the time the pathogenicity of this organism was still in doubt, the official report noted: "While an unidentified virus seems the most likely etiologic agent, the unusual prevalence of *Giardia lamblia* cysts in stools of patients seems worthy of record."

Between 1970 and 1982, at least 50 waterborne outbreaks resulting in approximately 20,065 cases of giardiasis have been reported in the United States (Craun 1979, USPHS 1980, 1981, 1982a, 1982b). Most of the water supplies involved in the outbreaks depended upon surface water treated solely by disinfection. Cysts in these outbreaks have been linked to humans and animals.

The earliest documented report of epidemic giardiasis in the U.S. came from Aspen, CO (Moore et al. 1969). A survey of skiers who were in Aspen during the 1965-66 ski season showed that at least 123 of 1,094 skiers exhibited symptoms of giardiasis. Examination revealed sewage contamination of wells that provided part of the city's water supply.

Brady and Wolfe (1974) reported five cases of giardiasis epidemiologically linked to a contaminated underground cistern on a farm in Tennessee. Rendtorff (1975) criticized the report by Brady and Wolfe because they reported finding *Giardia* trophozoites in the cistern water.

Witherell and Herbert (1977) discussed an epidemiological investigation of two giardiasis outbreaks in Vermont in 1974. In both outbreaks, water was considered to be the most likely vehicle of transmission since both water systems used surface water from unprotected and populated watersheds. Chlorination was the only treatment that the water had received.

Shaw et al. (1977) reported a community-wide outbreak of giardiasis in residents of Rome, NY. Between November 1974 and June 1975, 350 laboratory confirmed cases of giardiasis were detected. The city's water supply was surface water that was disinfected by chlorination (by chloramine) only. This study was the first in which a *Giardia* cyst was found in municipal water. In this study, 12 specific-pathogen-free beagle puppies were given aliquots of raw filter sediment. Two of the experimental dogs became cyst-positive for *Giardia*.

Lopez et al. (1980) investigated another outbreak of giardiasis in a municipal water supply in Berlin, NH, in 1977. The local hospital reported 213 predominantly symptomatic cases of giardiasis, 13% of which required hospitalization. In this study deficiences were noted in the construction of a wall separating the filtered and unfiltered water, and *Giardia* cysts were recovered from both types of water.

In 1976, an outbreak of giardiasis occurred in Camas, WA (Kirner et al. 1978, Dykes et al. 1980) with 128 confirmed cases of giardiasis. *Giardia* cysts were identified in water samples taken from streams entering the city water supply and from two storage tanks containing chlorinated water (1.2 mg/L with a detention time of 90 minutes). In this case, deficiencies in the chlorination and filtration equipment were noted.

Leakage of sewage into the city water supply, filter breakthrough, and an interruption of chlorination all were believed to be involved in a waterborne outbreak in the community of Vail Village, CO, in 1978 (USPHS, 1978). An estimated 5,000 cases of disease occurred. Giardia cysts were identified in water in the district serviced by the broken sewer line.

Weniger et al, (1983) reported an outbreak of gastrointestinal illness in Red Lodge, Montana which affected an estimated 780 people in the summer of 1980. This estimate was based on an attack rate of 33% in urban residents and 15% in rural residents. Giardia lamblia was found in the stools of 51% of the 47 people with a history of untreated gastrointestinal illness and in 13% of 24 of the stools from asymptomatic people. These investigators noted a bimodal curve with peaks in mid-June and mid-July. Each peak occurred approximately three weeks after an episode of heavy water runoff into the creek serving as the primary water source for the city. According to tests using standard methods of water analysis, the unfiltered creek water was inadequately chlorinated. No chlorine residuals were found at city taps and coliforms were cultured from the tapwater. Two large-volume water samplers of the EPA-type were installed at the treatment plant, but no Giardia cysts were identified in filtrates. Random fecal samples from 18 symptomatic people cultured at the CDC for Salmonella, Shigella, Campylobacter, Yersinia enterocolitica, pathogenic vibrios and enterotoxigenic E. coli were negative. However, one of four samples cultured by the Montana State Laboratory was positive for Campylobacter jejuni.

Tables III-1 and III-2 summarize the waterborne outbreaks of giardiasis in the U.S. from 1965 through 1977. Recent outbreaks of waterborne giardiasis are summarized in Table III-3. It should be noted that the number of cases in these tables represents cases of disease with Giardia-like symptoms, but not necessarily laboratory confirmed cases of Giardia cysts in the stool.

B. Sources of Giardia in Waterborne Outbreaks

The source(s) of cysts in the three major waterborne outbreaks of giardiasis are summarized in Table III-4. This will be discussed in detail below.

1. Fecal Contamination of Water by Humans

One of the first suggestions that water contaminated with human sewage was the source of cysts for an outbreak of epidemic giardiasis was a report by Moore et al. (1969). Epidemiologic data indicated that cross-connections between the water and sewage lines in Aspen, CO, allowed for cysts to enter the water supply.

An investigation of the episode of waterborne giardiasis that occurred in Berlin, NH, revealed that one of the streams that supplied water to the city was exposed to sewage discharge upstream. Furthermore, the watersheds were subject to human recreation but no sanitary facilities were provided (Lopez et al. 1980). Five beavers, five otters and four mink were collected from the Berlin watershed and examined for the presence of Giardia. All animals except for one beaver were negative for Giardia cysts and trophozoites. The infected

Table III-1 Waterborne Outbreaks of Giardiasis in United States

Location	Date	Cases	Water Supply[a]	Deficiency	Bacterial Quality of Water
Aspen, CO	Dec. 1965-Jan. 1966	123	M	Sewage contamination of wells; source of water was wells and surface supply with disinfection of each source.	Intermittent coliform contaminational of water distribution system.
Lookout Mt., CO	Aug. 1969	19	SP	Unknown.	Unknown
Idyllwild, CA	May-June 1970	34	M	Surface water source with filtration and disinfection; filters used intermittently.	One sample during outbreak showed >16 coliforms/100 mL; 7 samples in 6 months prior to outbreak and 5 samples in July 1970 were all negative; all distribution system samples.
Campground; Boulder Co., CO	May 1972	28	SP	Surface water with disinfection only; defective chlorinator.	Unknown
Resort; High Co., CO	May 1972	24	SP	Surface water with disinfection only; defective chlorinator.	Unknown
Camp; San Juan Area, UT	Sept. 1972	60	SP	Use of untreated surface water.	Unknown

continued-

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[a] Abbreviations used are:
M = Municipal
SP = Semi-public
I = Individual

Table III-1 - continued

Location	Date	Cases	Water Supply	Deficiency	Bacterial Quality of Water
Subdivision; Park Co., CO	Dec. 1972-Jan. 1973	12	M	Septic tank seepage into wells; no treatment.	Unknown
Lodge; Grand Co., CO	July 1973	16	SP	Use of untreated surface water.	Unknown
Farm; TN	Aug. 1973	5	I	Seepage from pit privy contaminated cistern.	Unknown
Essex Center, VT	Nov. 1973-April 1974	32	M	Surface water with disinfection only.	Raw water samples showed source to be contaminated, low coliform count after chlorination.
Danville Greet, VT	Dec. 1973-April 1974	20	M	Surface water with disinfection only.	Watershed survey revealed two homes with septic tank drainage into pond; no chlorine residual in distribution system.
Lodge; Grand Co., CO	June 1974	18	SP	Surface water with disinfection only.	Unknown
Meriden, NH	June-Aug. 1974	78	M	Surface water with disinfection only.	Unknown
Campers; UT	Sept. 1974	34	I	Use of untreated surface water.	Stream samples had mean fecal coliform count of 42/100 mL.

continued-

Table III-1 - continued

Location	Date	Cases	Water Supply	Deficiency	Bacterial Quality of Water
Rome, NY	Nov. 1974-June 1975	4800	M	Surface water with disinfection only.	Routine sampling of distribution system showed coliform levels to be less than 1/100 mL on 527 samples collected from Nov.-June; 4 samples in Feb. showed 20, 20, 30 & 40 coliforms/100 mL.
Campers; ID	Sept. 1975	9	I	Use of untreated surface water.	Unknown
Office & Residence Grand Co., CO	Feb. 1976	12	SP	Use of untreated surface water.	Twenty-three coliforms/100 mL.
Camas, WA	May 1976	600	M	Wells and surface water treated by pressure filtration and disinfection; deficiencies found in operation of filters and chlorinators.	Routine samples showed finished water met coliform standards.
Camp; Estes Park, CO	June 1976	27	SP	Surface water with disinfection only.	Water source contained less than 0.5 fecal coliforms/100 mL.
Berlin, NH	April 1977	750	M	Surface water with conventional treatment and surface water with pressure filtration and disinfection; deficiencies found in construction of gravity filter and operation of pressure filter.	Routine samples of distribution system were consistently negative.

continued-

Table III-1 - continued

Location	Date	Cases	Water Supply	Deficiency	Bacterial Quality of Water
Campground, UT	June 1977	770	SP	Use of untreated well water influenced by surface water.	Unknown
Hotel; Glacier Park, MT	July 1977	55	SP	Use of untreated surface water.	Unknown
W. Sulfur Springs, MT	July 1977	246	M	Surface water with disinfection only.	Unknown

Adapted from Craun (1979).

Table III-2 Annual Incidence of Waterborne Outbreaks of Giardiasis in the United States from 1965 to 1977

Year	Outbreaks	Cases
1965	1	123
1966	-	-
1967	-	-
1968	-	-
1969	1	19
1970	1	34
1971	-	-
1972	4	124
1973	4	73
1974	4	4,930
1975	1	9
1976	3	639
1977	4	1,058
Total	23	7,009

Adapted from Craun (1979).

Table III-3 Waterborne Disease Outbreaks by Etiology and Type of Water System from 1978 to 1981

	Public Water Systems				Private Water Systems		Total	
	Community		Noncommunity					
	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases
Giardia (1978)[a]	2	5,130	1	23	1	18	4	5,171
Giardia (1979)[b]	5	3,744	2	2,120	0	0	7	5,864
Giardia (1980)[c]	5	1,689	2	35	0	0	7	1,724
Giardia (1981)[d]	8	265	1	32	0	0	9	297
Total	20	10,828	6	2,210	1	18	27	13,056

[a] USPHS (1980).
[b] USPHS (1981b).
[c] USPHS (1982b).
[d] USPHS (1982c).

Table III-4 Source of *Giardia* Cyst Contamination in Waterborne Epidemics of Giardiasis

Source of Water Contamination	1975 Rome, NY	1976 Camas, WA	1977 Berlin, NH
Sewage cross-connection	No	No	No
Human	Undetermined but possible	Unlikely	Likely
Animal	Undetermined but possible	Likely	Possible
Beaver		3/7[a]	1/5
Otter			0/5
Mink			0/4
Porcupine			0/1
Coyote		0/1	
Opossum		0/1	
Opossum		0/2	
Nutria		0/1	

[a] Number positive/number examined.

Adapted from Juranek (1979).

beaver was found to harbor *Giardia* trophozoites in the small intestines but no cysts were found in the bowel contents. It was unclear in the minds of these authors whether the beaver was a culprit or a victim, but it was considered more likely that humans were the source of *Giardia* cysts in this outbreak.

2. Fecal Contamination of Water by Animals

Dykes et al. (1980) implicated beavers as the probable reservoirs and source of *Giardia* cysts in the Camas, WA, *Giardia* epidemic. Unlike the Berlin, NH, or Aspen, CO (Lopez et al. 1980, Moore et al. 1969) outbreaks, no evidence of human trespassers in the watershed, sewage drainage into the water supply or cross-contamination by sewage lines could be found in Camas. Instead, three beavers (*Castor canadensis*) trapped in the watershed were found to be infected with *Giardia*. Cysts from these beavers were infective for specific-pathogen-free beagle pups. Because beavers were implicated as reservoirs for *Giardia*, a survey of commercially trapped beavers and other mammals from the state of Washington was undertaken (Frost et al. 1980). Mammals were trapped during the 1976-77, 1977-78 and 1978-79 trapping seasons. The results of this survey are presented in Tables III-5 and III-6. The mammals surveyed included beavers, muskrats, nutrias, mink, raccoons, river otters, bobcats, coyotes and lynx. Among these animals, only beavers and muskrats harbored *Giardia* cysts. The prevalence of *Giardia* in beavers and in muskrats (Table III-6) suggested that *Giardia* was a common parasite in these animals.

Table III-5 Parasite Findings

Animal	Parasites[a]	Number Trapped (Percent Giardia-infected) 1976/1977	1977/1978	1978/1979
Beaver	G,C,Tr	173 (6.3)	177 (6.8)	179 (19)
Muskrat	G,E,Ch,C, Tr,N,Tc	1	17 (35.2)	115 (42.6)
Nutria	C,Tr	1	2	5
Mink	Tr,N,Tc		5	7
Raccoon	C,N,Tc		4	24
River Otter	Tr	1	2	10
Bobcat	To			7
Coyote	To			2
Lynx	To			1

[a] Abbreviations used are:

G = *Giardia* sp.
C = coccidia
E = *Entamoeba muris*
Ch = *Chilomastix* sp.
Tr = trematode eggs
N = nematode larvae
To = *Toxocara* sp.
Tc = *Trichuria* sp.

Adapted from Frost et al. (1980).

Table III-6 Parasite Associations of Beaver and Muskrat

Parasites	Beaver Giardia Positive (n = 34)	Beaver Giardia Negative (n = 145)	Muskrat Giardia Positive (n = 49)	Muskrat Giardia Negative (n = 66)
Coccidia	11.8	19.3		
Trematode	11.8	15.9	14.3	22.7
Coccidia, trematode	8.8	6.9		
E. muris			22.4	10.6
E. muris & other parasites			22.4	13.6
Other trematode, coccidia, nematode & *Chilomastix*			22.4	7.5
No other parasites	67.6	57.9	18.4	45.5

Adapted from Frost et al. (1980).

Published reports demonstrating other animal species as reservoirs for Giardia do not exist. However, the work of Hewlett et al. (1982) and Davies and Hibler (1979) suggest that domestic animals such as dogs, cats and cattle could potentially serve as sources for Giardia cysts in waterborne as well as other outbreaks of giardiasis.

C. Environmental Distribution

1. Geographic Factors

a. Worldwide Distribution

There is little doubt that human infections with Giardia occur worldwide. Reports cited below place Giardia in all of the major climatic regions from the tropics to the arctic. Petersen (1972) summarized early reports of Giardia in various geographic locations. In his review, reports of giardiasis came from Norway, Sweden, Finland, Germany, Switzerland, Romania, Czechoslovakia, the U.S. and India.

Cases of giardiasis have been observed in Australia (Kerlin 1978, Jones 1980), Nigeria (Pugh et al. 1981), Canada (Croll et al. 1979), U.S.S.R. (Jokipii and Jokipii 1974), Tasmania (Goldsmid 1980), Colombia (Danciger and Lopez 1975), arctic Greenland (Babbott et al. 1961), England (Ormiston et al. 1942), Ireland (McGrath et al. 1940), South Africa (Freeman and Grunewald 1980) and Libya (Ghannoum et al. 1981).

Petersen (1972) stated that giardiasis in tropical and subtropical areas probably reflects poor hygienic conditions. This hypothesis was corroborated by Pugh et al. (1981) when they studied two villages in Nigeria. One village had a water shortage and one, located near a dam, had no water shortage. Giardia was the most common intestinal parasite of children in the village with the water shortage where lower hygienic conditions existed.

b. United States Distribution

Although the CDC does not require notification of cases of giardiasis, it is clear that the disease occurs in every state and territory of the U.S. Specific state and territorial surveys and outbreak investigations show that giardiasis occurs in Alaska, Arizona, Arkansas, California, Colorado, Delaware, District of Columbia, Florida, Guam, Hawaii, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Maryland, Minnesota, Mississippi, Missouri, Montana, New Hampshire, New Mexico, New York, Ohio, Oklahoma, Oregon, Pacific Trust Territory, Pennsylvania, Puerto Rico, South Carolina, South Dakota, Tennessee, Texas, Utah, Vermont, Virgin Islands, Virginia, Washington, West Virginia, Wisconsin, and Wyoming (Craun 1979, USPHS 1981b, 1982a, 1982b, Weiss et al. 1977, Murphy 1981, Walker et al. 1980, Elliot et al. 1981, Schmidt 1982, Killion et al. 1981).

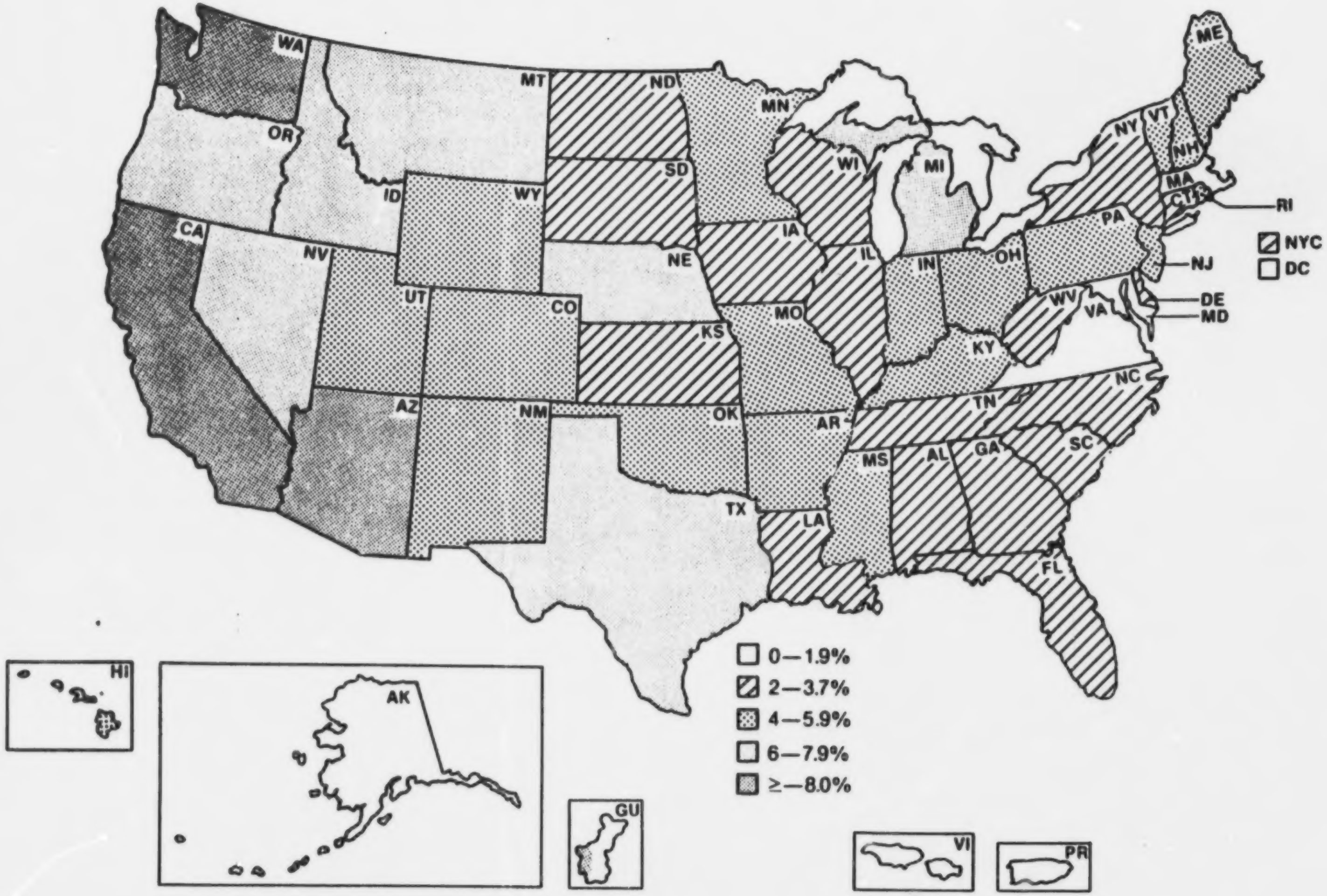
The distribution of Giardia in humans in the U.S is not limited to the states cited above. In the last CDC Intestinal Parasite Surveillance Summary (USPHS 1979a), Giardia was listed as the most commonly encountered pathogenic intestinal parasite in the U.S. While the CDC summary should not be construed as a random survey, it does compile the results of 332,312 stool specimens from patients submitted for parasitological evaluation in 1978 by 53 state and territorial public health laboratories. Of the 332,312 patient specimens submitted, 13,086 (3.9%) were positive for Giardia. Massachusetts submitted no stool specimen reports. For those states that reported, the percentage of Giardia-positive stools ranged from greater than 8% of the specimens from Arizona, California and Washington to 1.1% of the specimens from Virginia. For a summary of these findings, see Figure III-1.

In contrast to the above data from patients, Harter et al. (1982) found that 7.1% of 518 healthy children between one and three years old were positive for Giardia cysts.

2. Environmental Factors

a. Effects of Water Temperature on Giardia Cyst Survival

The effect of temperature on cyst survival is one of the few environmental parameters studied in detail for Giardia cysts. Bingham et al. (1979), using aliquots of a single batch of cysts isolated from a human, exposed cysts in tap water at -13, 8, 21, 37 and 100° C for various lengths of time and determined cyst viability. Viability was assessed using both eosin exclusion (theoretically denoting viable cysts) and in vitro excystation (Bingham and Meyer 1979, Bingham et al. 1979). For cysts incubated at 37° C, excystation reached a peak of from 9 to 10% by day 3 and dropped to nearly zero by day 6.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Adapted from USPHS (1979a) and Arkansas Department of Health (1978, 1979)

FIGURE III-1 PERCENTAGE OF STOOL SPECIMENS POSITIVE FOR GIARDIA LAMBLIA, 1978

By eosin exclusion, nearly 60% of the cysts from the same aliquot appeared viable on the first day and the percentage dropped slowly to zero by day 24. For cysts stored at 21° C, excystation reached a peak of nearly 40% on day 9; the level of excystation decreased gradually to zero by day 26. Eosin exclusion results at 21° C, which began at approximately 95% viability, decreased slowly during the same time period and indicated 100% death by day 32. Cysts stored at 8° C reached the highest percentages of excystation (60%); 10 to 60% excystment continued for the first 30 days of the experiment. Low levels of excystation (less than 5%) were observed for 77 days after the experiment began. At this same temperature, eosin exclusion tests of cysts indicated viability of from 80 to 95% during the 77-day period.

Bingham and co-workers (1979) noted that cysts subjected to boiling water were immediately rendered incapable of excystation. Studies in which cyst viability was promptly determined after cyst suspensions in water were frozen and thawed, indicated that the freezing process destroys most Giardia cysts. Fewer than 1% of the Giardia cysts survived freezing for 14 days. These authors speculated that the increased cyst survival in cold (but not frozen) water rather than warm water may have epidemiological significance in light of the outbreaks of giardiasis in such places as Camas, WA; Rome, NY; Berlin, NH; and Leningrad, U.S.S.R.

Grant and Woo (1978b) assessed the infectivity of G. simoni cysts from rats with cyst viability measured by eosin exclusion. In their experiments, feces from infected laboratory rats were suspended in 0.85% saline and divided into three aliquots. These aliquots were stored at 5, 24 and 36° C, respectively and eosin staining was attempted at 24-hour intervals. Approximately 85% of these cysts were able to exclude eosin on day zero. From these same suspensions, groups of rats were given 5,000 cysts at regular intervals and then examined for infection 14 to 28 days post-inoculation.

Grant and Woo (1978b) observed exponential declines in the number of cysts that excluded eosin at all temperatures. Within 24 hours after the cysts in saline were exposed to 36° C, only 27% were able to exclude eosin and no rats were infected. Cysts stored in saline at 24° C were infective to rats on day 1, when 69% of the cysts excluded eosin, but by day 2 the exclusion rate had dropped to 43% and no cyst-challenged rats were positive for *Giardia* trophozoites or cysts. Rats were infected with cysts stored at 5° C until day 4 when the percentage of eosin exclusion dropped to approximately 49%.

b. Other Factors that Affect *Giardia* Cyst Survival

Literature dealing with the effects of other environmental factors such as pH, desiccation, ultraviolet irradiation or hypertonic solutions on *Giardia* cyst viability are sparse.

The effect of chlorine concentration on *G. lamblia* cyst viability was tested under a variety of environmental conditions (Jarroll et al. 1981a). The criterion of cyst viability in these experiments was *in vitro* excystation as described previously by Bingham and Meyer (1979) and Bingham et al. (1979). The variables that they employed included water temperature (25, 15 and 5° C), pH (6, 7 and 8), chlorine-cyst contact time (10, 30 and 60 minutes) and chlorine concentration (1 to 8 mg/L). In the pH range studied, cyst survival in the presence of chlorine generally was observed to increase as the buffer pH increased. Water temperature coupled with chlorination proved to be important in cyst survival. At 25° C, exposure to 1.5 mg/L chlorine for 10 minutes killed all cysts at pH 6, 7 and 8. At 15° C, 2.5 mg chlorine/L for 10 minutes killed all cysts at pH 6, while at pH 7 and 8 small numbers of cysts (less than 0.8%) remained viable after 30 minutes, but not after 60 minutes. At 5° C, 1 mg chlorine/L for 60 minutes failed to kill all of the cysts at any pH tested. At this temperature, 2 mg chlorine/L killed all cysts after 60 minutes at pH 6 and 7, but not at pH 8. A chlorine concentration of 4 mg/L killed all the cysts at all three pH values after 60 minutes, but not after 30 minutes. A chlorine concentration of 8 mg/L killed all *Giardia* cysts at pH 6 and 7 after contact for 10 minutes, and at pH 8 after 30 minutes. This study pointed up the role of temperature, pH and chlorine in the halogen treatment of drinking water to destroy *Giardia* cysts.

Craft (1982) reported that *Giardia* cysts used in his rat infection experiments remained infective for one year when stored in liquified feces at 4° C. He noted that a rapid loss of infectivity occurred when the cysts were stored at 4° C in Hanks balanced salt solution.

The only report in the literature dealing with the effects of desiccation on *Giardia* cysts stated briefly that these cysts were highly sensitive to drying (Cerva 1955). Although Cerva gave no details on how he arrived at this conclusion, the conclusions he reached in the same paper concerning the effect of various disinfectants on Giardia cysts were based on eosin exclusion determinations of cyst viability.

D. Summary

Giardiasis, like many other gastrointestinal diseases, is acquired by the fecal-oral route. Epidemiological evidence exists that suggests transmission may at times be venereal (especially among homosexuals), foodborne or waterborne. In day-care centers, transmission appears to be directly fecal-oral and often due to failure to maintain sanitary conditions among groups of young children and their attendants.

In waterborne outbreaks, human and animal feces have been implicated as the source of human infections.

Giardia infections are worldwide in distribution in both humans and lower animals, and Giardia is the most commonly reported pathogenic intestinal parasite in humans in the U.S. An important environmental factor which may be involved in waterborne outbreaks is water temperature. Giardia cysts remain viable in water at 8° C for up to 2 months while at 21° C they are viable for less than 1 month and at 37° C they survive for less than 1 week. Boiling water kills Giardia cysts immediately but freezing and thawing may allow some cysts to survive for several days. The role of temperature, pH and chlorine demand in the halogen treatment of drinking water is also discussed.

IV. HEALTH EFFECTS IN ANIMALS

A. Acute Disease

1. Symptomatology

The signs of symptomatic giardiasis in animals, in general, appear to be similar to signs of the disease in humans (see Section V). These signs include diarrhea, anorexia, weight loss, vomiting and steatorrhea (Panigrahy et al. 1978, 1979, Sachs 1963, USPHS 1979b, Brightman and Slonka 1976, Bemrick 1961, Shelton 1954a, 1954b).

2. Pathology

In parakeets giardiasis has been associated with high morbidity and mortality (Panigrahy et al. 1978, 1979, 1981, Hirai et al. 1980). Infected birds may exhibit diarrhea, decreased intake of food and water and debility. Mortality among such parakeets may range from 20 to 50%. In fatal cases, Panigrahy et al. (1978) examined smears from the intestinal mucosa of the birds and found a heavy *Giardia* trophozoite burden with mortality occurring even in the absence of bacterial, chlamydial or viral infections.

In chinchillas, Sachs (1963) found *Giardia* trophozoites in the blood of an infected animal at autopsy. Shelton (1954a, 1954b) observed signs similar to those found in parakeets when he infected chinchillas with *Giardia*. He noted that not all of the chinchillas became symptomatic, but that three of the four that did become symptomatic died. Among the symptoms, he observed diarrhea, anorexia and occasionally cessation of fecal elimination. In the three fatal cases, necrotic foci in the large bowel, fatty changes in the liver, hemorrhagic gastritis and emaciation were evident. In two of the fatal cases *Pseudomonas* was isolated from the heart and kidneys. In the third case the tapeworm *Hymenolepis* was found in the intestine.

In mice inoculated with *Giardia* cysts, the number of trophozoites and cysts reached a peak within 5 to 14 days. The infection was observed to clear spontaneously in most animals within 21 to 28 days. Although, in the mouse, maximum parasite burden was independent of the number of cysts inoculated, the time required to attain the maximum parasite burden was dependent upon the size of the inoculum (Roberts-Thomson et al. 1976).

Belosevic and Faubert (1983) administered from 10 to 10^5 *G. muris* cysts to CD-1 Swiss mice by gastric intubation. To ensure that the mice were free from *Giardia* infection all were treated with Metronidazole (10 mg/mouse/day). The mice feces were examined for cysts once each week after the treatment. The investigators' results suggest that the appearance of cysts in the feces of mice infected with from 10 to 100 cysts required five days, while cysts appeared in the feces of mice infected with 10^5 cysts after only two days.

Several reports, supported by light micrographs, have appeared in the literature suggesting that *Giardia* trophozoites penetrate the mucosal cells of the intestine as well as extraintestinal tissues in rodents (Sachs 1963, Herman 1943, Tumka 1972, Akimova and Solov'ev 1973).

Household pets have also been observed to show signs of Giardia infection. Five cases of clinical giardiasis in cats have been reported by Brightman and Slonka (1976). In these five cases, they noted signs which included weight loss, mucoid, soft stools and the presence of split and unsplit fats in the feces. Dogs less than one year old appear to be more likely to have symptomatic giardiasis than older dogs (Bemrick 1961). Signs in canines include diarrhea with mucus and fats, listlessness and anorexia. In puppies, severe giardiasis may result in complications ranging from growth retardation to death (Craige 1948).

The signs and clinical picture of symptomatic giardiasis in apes was observed at the Kansas City, MO, zoo (USPHS 1979b). The signs exhibited by the infected apes were similar to those seen in their human attendants who also contracted the disease. Although no fatalities were reported, all patients suffered from loose stools, diarrhea and vomiting. It is unknown if giardiasis in apes and monkeys is a self-limiting disease since all animals received chemotherapy.

B. Chronic Disease

Few reports of chronic giardiasis in animals appear in the literature. However, indications from the mouse model system studied by Roberts-Thomson et al. (1976) and Roberts-Thomson and Mitchell (1978) indicate that some strains of mice are incapable of spontaneously clearing Giardia infections.

1. Symptomatology

In the mouse model system of Roberts-Thomson et al. (1976), mice infected with *G. muris* exhibited impaired weight gain and diarrhea. No other overt signs were reported.

2. Pathology

Upon microscopic examination of *Giardia*-infected mouse intestinal tissues, Stevens and Roberts-Thomson (1978) observed that there was a decreased crypt-to-villus ratio and increased numbers of inflammatory cells present in the lamina propria of mice infected with *G. muris*. Roberts-Thomson and Mitchell (1978) and Roberts-Thomson et al. (1976) associated the ability or inability of mice to clear *Giardia* infections with strain differences among mice. These authors found that mice of strain C3H/He produced cysts at a relatively constant rate during their 10-week study while mice strains CBA/H, A/J, SJL, BALB/c and DBA/2 showed a steady decline in cyst production after reaching an initial peak in cyst production. All of these strains, except for the C3H/He mice, showed greater resistance to reinfection with *G. muris*.

Studies by Roberts-Thomson and Mitchell (1978) and Boorman et al. (1973) demonstrated the inability of athymic (nude) mice to resolve *Giardia* infections spontaneously or to resist reinfection. Such immunosuppressed mice whose lymphoid systems were reconstituted with lymphoid cells from syngeneic, thymus-intact mice exhibited a progressive reduction in cyst numbers (Roberts-Thomson and Mitchell 1978). Furthermore, it was observed that if the thymus-intact mice used as lymphoid donors had been previously exposed to Giardia, then clearing of the infection in the recipient mice was accelerated (Roberts-Thomson and Mitchell 1978).

Although some strains of mice do apparently clear Giardia infections spontaneously, as determined by cessation of cyst production, Stevens and Frank (1978) observed pregnant mice, apparently cleared of Giardia infections, and reported that cyst production started again just prior to delivery. Stevens and Frank (1978) proposed that sensitized lymphoid cells migrated from the intestine into mammary gland sites during lactation.

Underdown et al. (1981) continued the study of chronic infections in C3H/He mice. They found that C3H/He mice were able to resist challenge infection after the administration of antigiardial chemotherapy even though C3H/He mice were incapable of clearing primary infections. Furthermore, mouse mothers were able to protect their suckling young from a challenge infection. Underdown et al. (1981) were unable to relate the ability to eliminate primary infection or resist challenge infections to serum concentration of IgA. As a result of this study, Underdown et al. suggested that the inability of C3H/He mice to clear a primary infection with Giardia may be due to the ability of C3H/He mice to neutralize molecules produced by G. muris which inhibit host effector response.

C. Summary

Signs of giardiasis in animals appear to be similar to those in humans. They include diarrhea, anorexia, weight loss and steatorrhea. As in humans, the disease resolves spontaneously in most animals but may be chronic or fatal in some.

V. HEALTH EFFECTS IN HUMANS

A. Acute Disease

Although it occurs less frequently than asymptomatic giardiasis, symptomatic giardiasis is commonly encountered (Jokipii 1971, Wolfe 1979, Degremont et al. 1981) and is often associated with epidemics (see Section III). Immunocompromised individuals (Ament and Rubin 1972) and individuals with achlorhydria (Yardley et al. 1964, Gianella et al. 1973) have been reported to be particularly susceptible to symptomatic *Giardia* infection.

The incubation period of giardiasis (based on cyst detection in the feces) ranged from 9 to 22 days with an average of 13.1 days (Rendtorff 1954, Rendtorff and Holt 1954). Walzer et al. (1971) and Wolfe (1979) reported that the mean time period between infection and the onset of acute disease was 12 to 15 days (ranging from 1 to 75 days) based on data from travelers to the U.S.S.R.

The duration of acute clinical illness may vary greatly. Smith and Wolfe (1980) stated that in some patients, symptoms lasted for only 3 or 4 days while in others the symptoms lasted for months. In general, however, patients commonly resolve their infections spontaneously and acute disease lasts from 1 to 4 weeks.

The only controlled study to determine the number of cysts required to elicit an infection in humans was performed by Rendtorff (1954). In these experiments, male volunteers were fed human-source Giardia cysts contained in gelatin capsules. Cyst dosages ranged from 1 to 1 x 10^6 cysts per capsule. Rendtorff's experiments showed that ten or fewer cysts could produce human infection (determined by observing presence of Giardia in fecal smears) see Table VII-1 in Section VII for greater detail. Rendtorff's failure to infect five men with a dose of one cyst may have been due to feeding these volunteers inactive cysts since he did not determine cyst viability in any of these experiments. None of these volunteers exhibited symptoms that Rendtorff could associate directly with the Giardia infection.

Fatal giardiasis accompanied by organic lesions and steatorrhea was reported by McGrath et al. (1940) in Ireland. Giardiasis has been reported as a cause of death in four cases in recent years in this country (USPHS 1982a).

1. Symptomatology

Numerous signs and symptoms have been associated with giardiasis in humans. Among the more common signs and symptoms are diarrhea, abdominal pain and distress, flatulence, malodorous stool, weight loss, vomiting and rarely death (Wolfe 1979, Degremont et al. 1981, Osterholm et al. 1981, USPHS 1982a). In a recent study of 158 patients with giardiasis at the Swiss Tropical Institute (Degremont et al. 1981), 115 patients with Giardia cysts in their feces were asymptomatic while 43 (27%) exhibited symptoms. Of the 43 symptomatic cases, 36 experienced diarrhea, 17 had abdominal distress, 12 had flatulence and 6 experienced vomiting.

A similar distribution of symptoms was observed in a foodborne outbreak of giardiasis in which 29 people experienced symptoms of the disease (Table V-1) (Osterholm et al. 1981). Wolfe (1979) summarized the signs and symptoms of 32 individuals returning from the Soviet Union and 105 State Department cases (Table V-2). Of the State Department cases, 6.7% were considered to have severe, incapacitating symptoms including marked weight loss, watery diarrhea and fatigue; while 13.3% had no symptoms.

2. Pathology

Carroll et al. (1961) linked a case of uveitis in a 9-year-old female to Giardia infection. Although the authors were unable to show a direct connection between the uveitis and giardiasis, the uveitis steadily improved and the shedding of Giardia cysts stopped following antigiardial chemotherapy. Giardiasis has also been associated with hypersensitivities in a 16-year-old male (Harris and Mitchell 1949). Both the patient's urticaria and his giardiasis disappeared following quinacrine therapy.

In a more recent case report by Weisman (1979), a female was treated for an urticaria problem of 19 months duration. This same patient was cyst positive for Giardia. Following the treatment of Giardia, the urticaria disappeared and did not recur during a 15-month follow up. The case physician, however, was unable to present an obvious explanation for the association and suggested that their association was simply coincidental.

An intolerance to mammalian meat was noted in a 29-year-old male physician with giardiasis (Halstead and Sadun 1965). A record of the

Table V-1 Frequency of Symptoms in 29 Cases of Clinical Giardiasis among Employees of the Goodhue, Minnesota, Public Schools, 1979

Symptom	No. of Cases[a]
Diarrhea	29 (100)
Fatigue	28 (97)
Abdominal cramps	24 (83)
Bloating	23 (79)
Malodorous stool	23 (79)
Flatulence	22 (76)
Weight loss[b]	17 (59)
Fever	6 (21)
Vomiting	5 (17)

[a] Figures in parentheses denote percentages.
[b] The average weight loss was 4.2 kg.

Adapted from Osterholm et al. (1981).

Table V-2 Clinical Features of 32 Individuals Recently Returned from the Soviet Union with Proven Giardiasis Compared with 105 State Department Cases with Undetermined Duration of Proven Infection

Symptom	State Dept. % (n=105)	USSR % (n=32)
Flatulence	46.7	56.5
Foul Stool	44.8	52.2
Cramps	32.4	59.4
Distention	31.4	--
Anorexia	20.0	56.2
Nausea	20.0	59.4
Weight Loss	18.1	--
Belching	15.2	30.4
Heartburn	14.3	--
Headache	11.4	--
Constipation	11.4	--
Vomiting	4.8	34.4
Fever	3.8	17.4
Chills	2.9	--
Diarrhea	62.9[a]	71.9
Blood in Stool	0	0
Mucus in Stool	3.8	4.4
Fatigue	28.6	87.5

[a] 52.4% had soft or mushy stools; 10.5% had watery stools.

Adapted from Wolfe (1979).

patient's diet and symptoms was kept during the course of his infection. Halstead and Sadun observed moderate to severe intestinal symptoms in the patient only when he had meals which included mammalian meat. Swordfish, shrimp, chicken and vegetables did not precipitate a reaction. The hypersensitivity to mammalian meat was relieved after treatment of the giardiasis.

Goobar (1977) examined 66 children younger than 16 who had symptoms of giardiasis and found inflammation of the synovial membranes of major joints in 42 of them and *Giardia* cysts in the feces of 62. Intestinal symptoms characteristic of giardiasis were also reported. Following quinacrine or metronidazole therapy, the intestinal and synovial symptoms abated.

Malabsorption and celiac-like syndrome have been associated with more severe *Giardia* infections (Veghelyi 1939, Hoskins et al. 1967, Morecki and Parker 1967, Barbieri et al. 1970, Brandborg 1971). Carswell et al. (1973), however, were unable to connect these severe conditions with giardiasis. They found that the incidence of *Giardia* infection in children with and without celiac syndrome was essentially the same. In their study, patients with giardiasis had less severe histological changes than those without the disease. A more recent study by Duncombe et al. (1978) draws a positive correlation between the severity of the villus atrophy and the severity of the diarrhea in patients with *Giardia* infections.

B. Chronic Disease

1. Symptomatology

Wolfe (1979) described non-acute and chronic patients as those who present with recurrent persistent brief episodes of loose, foul-smelling stools which may be yellowish and frothy in appearance. Furthermore, these symptoms may be accompanied by distension, foul flatus, anorexia, nausea and uneasiness in the epigastrium as frequent complaints. Wolfe (1979) stated that in some cases, these symptoms may persist for years, but that in the great majority of cases the parasite and symptoms disappear spontaneously.

Overton and Hertko (1963) reported a case of intermittent fever apparently associated with giardiasis that lasted for 11 years. This occurred in a 39-year-old female from the Midwest. From 1950 to 1960 the patient was seen by her physician on numerous occasions in which she complained of fever and arthralgia of the wrists. Laboratory tests in 1950 and again in 1960 failed to detect any physiological, anatomical or microbiological abnormalities. In 1961, laboratory tests were again negative for everything except large numbers of Giardia cysts in the stool. Following quinacrine therapy, the fever subsided in three days and excretion of cysts stopped within five days. During the 16-month observation period that followed, no recurrence of fever or other symptoms occurred.

Asymptomatic giardiasis appears to occur more frequently, and may be epidemiologically more significant, than symptomatic giardiasis. Individuals with asymptomatic Giardia infections are much less likely to be detected or to seek treatment than those with symptomatic infections and, therefore, are more likely to serve as carriers or disseminators of the disease (Meyer and Jarroll 1980). Subacute or carrier infections may last for months or years (Smith and Wolfe 1980, Danciger and Lopez 1975).

2. Pathology

Few reports on the pathology of chronic giardiasis are to be found in the literature. However, Hoskins et al. (1967) studied five cases of giardiasis associated with intestinal malabsorption. In one of these five cases, the patient had a sprue-like syndrome with IgA deficiency, vitamin B_{12} malabsorption, and recurrent diarrhea which persisted for 25 years. Early in the course of the disease, the diarrhea episodes occurred at intervals of a year or more, but in the three years prior to his death at age 47, the episodes were more or less continuous. The episodes consisted of 15 to 20 foul-smelling stools daily, anorexia, vomiting, nausea and abdominal cramps. Histology showed abnormal villus architecture. Although Giardia trophozoites were first found in 1956, 3 years after the episodes began, no atabrine therapy was ever administered to the patient, who subsequently died in 1963. This patient's death was not attributed to giardiasis directly.

Hoskins et al. (1967) also reported a case of giardiasis in a female college student whose symptoms persisted for 5 months. In this case, no villus atrophy was noted but there was marked round cell infiltration in the lamina propria.

C. Immunity

Although the data available to date on the nature of the human immune response to Giardia infection are rather limited, it seems clear that both humoral and cellular responses are involved. The repeated observations of spontaneous recovery from this disease, even in the absence of treatment (Rendtorff 1954), and of chronic diarrhea in those with immune deficiencies (Ament and Rubin 1972) support the role of an immune response in human giardiasis.

1. Humoral

Several workers have determined the total immunoglobulin levels in individuals with giardiasis. In their series of patients, Jokipii and Jokipii (1982) noted that the total immunoglobulin levels fell within normal limits. Radulescu et al. (1974) reported the results of a study of sera from 35 giardiasis patients. Thirty-two of the 35 sera proved positive for Giardia in an indirect immunofluorescence test; the immunoglobulin levels of all of these patients were either normal or elevated. In contrast, the immunoglobulin levels in the three immunofluorescent-negative sera were all lower than normal.

Observations on the serum immunoglobulin levels of IgG, IgM, IgA, IgE, and IgD also have been reported in patients with giardiasis. Levels of IgM not significantly different from those in normal patient controls have been described by Naik et al. (1979b), Jones and Brown (1974), Zinneman and Kaplan (1972) and Ridley and Ridley (1976). On the other hand, Jokipii and Jokipii (1982) reported that their patients with recently acquired infections had slightly elevated IgM levels.

Most of the workers who have studied serum IgG levels in giardiasis patients (Zinneman and Kaplan 1972, Jones and Brown 1974, Ridley and Ridley 1976, Jokipii and Jokipii 1982) report normal levels; Naik et al. (1979b) found serum IgG increased. Serum levels of IgA have been measured in patients with giardiasis by a number of workers (Jokipii and Jokipii 1982, Naik et al. 1979b, Ridley and Ridley 1976, Jones and Brown 1974, Zinneman and Kaplan 1972) and found to be within normal limits.

Only one study (Jokipii and Jokipii 1982) seems to have been directed at determining serum IgD levels in giardiasis. Although the mean serum concentration of IgD in 105 unselected patients with symptomatic Giardia infection was considered to be in the normal range, the concentration of IgD in those patients who had been losing weight rapidly was significantly lower than those of either the healthy controls or the other giardiasis patients.

With reference to serum IgE levels in giardiasis, there is a consensus among all workers who have reported on the subject (Geller et al. 1978, McLaughlan et al. 1974, Jones and Brown 1974, Brown et al. 1973). They agree that serum levels of IgE in giardiasis do not differ from those of normal controls.

In addition to investigating the levels of individual immunoglobulins, several workers have reported on studies aimed at demonstrating serum antibodies in patients with giardiasis. Ridley and Ridley (1976), for example, employed an immunofluorescence test to demonstrate anti-*Giardia* activity (IgG could be detected, IgA and IgM could not) in 32 of 36 cases of giardiasis with malabsorption; in neither of two cases of giardiasis without malabsorption and in none of 17 control patients with neither malabsorption nor giardiasis. Positive test results were noted in 10 of 34 cases of malabsorption without evidence of *Giardia*.

Roberts-Thomson and Anders (1981) observed levels of both IgA and IgG, in 22 infected patients to be higher than those in patients with previous infection and in adult and juvenile controls. Serum IgG levels fell relatively rapidly after cure, while serum IgA levels remained high. Smith et al. (1981a) demonstrated IgA antibody to *Giardia* in an enzyme-linked immunosorbent assay in 48 of 59 giardiasis patients and 2 of 17 controls.

Several groups of workers (Radulescu et al. 1974, Visvesvara and Healy 1979) noted anti-*Giardia* antibody in the serum of patients. Utilizing an anti-human fluorescein-labeled globulin as an indicator did not permit identifying the immunoglobulin class involved.

Secretory immunoglobulins in giardiasis have been studied by several groups of workers. Zinneman and Kaplan (1972) and Popovic et al. (1974) reported lower IgA concentrations in duodenal secretions of giardiasis patients than in those of controls. Although Naik et al. (1979b), Jones and Brown

(1974) and McClelland et al. (1973) could not verify the secretory IgA observation, the latter workers did note higher secretory IgG levels in patients than in controls; the difference was unexplained. Jones and Brown (1974) and Popovic et al. (1974) noted normal levels of IgM in intestinal fluids of patients with giardiasis.

A few workers have studied plasma cell populations in the lamina propria of patients with giardiasis, with conflicting results. Ridley and Ridley (1976), for example, noted an increased IgM cell fluorescence in the lamina propria, but no increase in either IgA or IgG fluorescence. Thompson et al. (1977) studied the production by jejunal immunocytes of these same three classes of immunoglobulins. Three cases showed increased IgM cells, seven cases showed increased IgM and IgA cells, one case showed increased IgA and IgG cells, and one case showed increased IgM, IgA and IgG cells. On the basis of these data and the data of Ridley and Ridley (1976), these authors suggested that the early immune response in the lamina propria to *Giardia* infection may be restricted to IgM, followed later by IgA and IgG. Popovic et al. (1974) also studied IgA- and IgM-producing cells in jejunal mucosa in giardiasis and noted a high percentage of cells containing IgM and a low percentage of cells containing IgA.

2. Cellular

Studies of cell-mediated immune response to *Giardia* infections in humans, in contrast to studies of humoral responses, are relatively few in number. A primary reason for this paucity of information may be the fact that obtaining cellular material, particularly from human small intestines, is more difficult than obtaining serum.

Smith et al. (1982) have demonstrated human peripheral blood mononuclear cells to be spontaneously cytotoxic for *G. lamblia* trophozoites. The fact that *Giardia* cytotoxicity could be inhibited by adding *Trichomonas vaginalis* suggested to the authors that the observed cytotoxicity was a host defense mechanism directed against extracellular protozoa generally. Although the precise mononuclear cells involved were not determined, their studies indicated that monocytes or macrophages, or both, are more likely to be involved than granulocytes or lymphocytes.

Intraepithelial lymphocyte counts in giardiasis were reported for children by Ferguson et al. (1976) and for adults by Wright and Tomkins (1977). A study of the biopsies of three of nine infected children were higher than the normal controls; the other six fell within the normal range. In the study of adults, patients with giardiasis and normal intestinal absorption had intraepithelial lymphocyte counts similar to those of control patients who had lived and traveled in tropical areas. It should be noted that the counts in both groups were higher than those observed in controls from temperate areas. In cases of giardiasis and malabsorption of one to three substances, the counts were significantly higher than those of the "tropical" controls and those of patients with giardiasis and normal absorption. With few exceptions, the counts declined after treatment.

Naik et al. (1979a) reported testing cellular immune function in ten patients with giardiasis, using intradermal tests for immediate (type I) and delayed (type IV) hypersensitivities; non-*Giardia* antigens were employed. The reactions of the giardiasis patients to the antigens tested were similar to those of the controls. The authors suggested that it would be of interest to examine these cell-mediated responses to specific *Giardia* antigen.

It has been suggested (Lancet 1982) that in giardiasis, humoral mechanisms may be more effective in preventing infection than in eradicating it, and that T lymphocytes may be important in the final (clearance) stage of the infection.

3. Diagnostic Tests

The development of serologic tests for the diagnosis of this disease was impeded, until recently, by the unavailability of methods for the culture of *Giardia* trophozoites. The present availability of axenic cultures of several isolates of *Giardia* trophozoites of the *G. duodenalis*-type has made possible the serologic studies described below because *G. duodenalis* is the morphological type considered by Filice (1952) to include *G. lamblia*.

Visvesvara et al. (1980) reported the use of the indirect immunofluorescence test in demonstrating titers in 29 of 30 patients with symptomatic giardiasis. *Giardia lamblia* trophozoites, grown axenically, were used as antigen and no cross- reactivity was observed between this antigen and *Entamoeba histolytica*, *Trichomonas vaginalis* or *Escherichia coli*. No overlap in titer occurred in 19 healthy control subjects. Ridley and Ridley (1976), Wright et al. (1977b), Lopez-Brea et al. (1979) and Radulescu et al. (1974) have also reported their experiences with the immunofluorescence test.

Vinayak et al. (1978), using Giardia cysts removed from human feces, reported the ability to detect serum antibodies by immunodiffusion. The lack of cysts derived from cultured trophozoites makes the question of cross reactivity with other fecal organisms a possibility.

Roberts-Thomson and Anders (1981), in a preliminary report, described the use of a solid-phase radioimmunoassay to detect serum antibodies to G. lamblia. Trophozoites of G. lamblia and G. muris were both used successfully as antigen.

Smith et al. (1981a) reported the development of a sensitive enzyme-linked immunosorbent assay employing axenically cultured G. lamblia trophozoites. They demonstrated circulating IgG in 81% of 59 symptomatic giardiasis patients. Two of the 17 uninfected control patients had circulating IgG antibody against Giardia.

Craft and Nelson (1982) reported success in identifying G. lamblia fecal antigen, using a counterimmunoelectrophoresis test, in 65 of 66 patients. They reported that the test appears to be as sensitive and as reliable as the combined examination of feces and duodenal fluid. Craft (1982) also reported using this method successfully in detecting G. lamblia in rats. At this writing, other workers had not published their experience with this method.

D. Summary

Symptomatic giardiasis, although less common than asymptomatic infection, occurs frequently and results in diarrhea, flatulence, abdominal pain, weight loss and vomiting. Severe disease may result in malabsorption or growth retardation, but rarely death. Diarrhea symptoms have been related to abnormal villus architecture. Chronic giardiasis appears to be infrequent, but when it occurs it may persist for years.

Data on the nature of human immune response to giardiasis are somewhat limited but suggest that both humoral and cellular responses are present. Research on serological tests for giardiasis appears to be advancing. Reports of success with immunofluorescence tests and enzyme-linked immunosorbent assays have appeared in the recent literature, but the development of a commercially available diagnostic test is still awaited.

VI. MECHANISM OF DISEASE

One of the most common symptoms associated with symptomatic giardiasis is diarrhea (Degremont et al. 1981, Osterholm et al. 1981, Wolfe 1979). The severity of the diarrhea has been positively correlated with the severity of changes in the villus histology (Duncombe et al. 1978). In the latter study, 17 patients with giardiasis were examined. Two of these patients, one of whom had a concomitant infection with Strongyloides, were asymptomatic. The villus architecture in both of the asymptomatic cases was normal. Diarrhea was graded according to stool frequency; it was considered mild if there were less than three stools per day, moderate if between three and six stools, and severe if there were more than six stools per day.

Villus architecture was assessed by histological observations of biopsies taken at the ligament of Treitz. The slides were read randomly and graded on a visual basis by noting the crypt-to-villus ratio, degree of inflammatory infiltrate and epithelial cell height.

Villus architecture was normal in four patients and subtotal villus atrophy was noted in one patient. In eight patients villus atrophy ranged from mild to severe. Diarrhea was reportedly mild in eight cases, moderate in five cases and severe in two cases. When the severity of the villus atrophy was compared with the severity of diarrhea, Duncombe et al. (1978) observed that the more severe the villus atrophy, the more severe the diarrhea. Furthermore, after antigiardial chemotherapy there was a positive correlation between recovery of the villus architecture and the disappearance of diarrhea.

Since no published reports were found to show that Giardia are toxigenic, it remains unknown whether liberation of a toxin could account for the villus atrophy observed.

Infections with Giardia frequently cause steatorrhea (fatty diarrhea) as a result of fat malabsorption (Saha and Ghosh 1967, Hoskins et al. 1967). Several mechanisms have been suggested to account for absorption abnormalities, including mechanical blockage of mucosal surfaces (Veghelyi 1939), functional mucosal changes brought on by invading trophozoites (Morecki and Parker 1967), mucosal damage from inflammation in the absence of invasion (Yardley et al. 1964), associated bacterial overgrowth (Tomkins et al. 1978, Tandon et al. 1977), deconjugation of bile acids (Tandon et al. 1977) and interference with lipolysis (Smith et al. 1981b). Experimental evidence for only the latter mechanism has been found in the literature.

Smith et al. (1981b) used Giardia trophozoites originally isolated from a human and cultured in vitro to assess the effect of Giardia on bile acids and lipolysis. In the deconjugation experiments, Giardia trophozoites were incubated with glycodeoxycholate, glycochenodeoxycholate and glycolate. Thin-layer chromatography of the resulting medium showed no liberated deconjugated bile acids. This was in direct contrast to duplicate experiments in which the bacterium Bacteroides fragilis did liberate deconjugated bile acids.

Smith et al. (1981b) further attempted to show that sonicates of Giardia trophozoites inhibited lipolysis. In this system, hydrolysis of tributyrylglycerol by porcine pancreatic lipase was decreased by two thirds from the controls when sonicates of Giardia trophozoites were added. It was pointed out in this paper that the amount of Giardia protein employed for the inhibition assays might not reflect the amount actually present in the intestinal lumen of patients.

Anand et al. (1980) used transport studies in segments of rat intestines to determine the effect of Giardia trophozoites from humans on transport of glucose, glycine and potassium. These authors noted there was a significantly lower transport of glucose and glycine in intestines from infected rats as opposed to non-infected rats. In contrast, no significant difference was observed in the transport of potassium. Anand et al. (1980) suggested that the presence of Giardia interfered with the active transport of glucose and glycine but not with the passive transport of potassium. They postulated that the defect in the active transport mechanism of the enterocyte could be due to structural damage of the substrate carrier or an alteration in cell maturation due to Giardia. No attempt was made to quantitate the number of Giardia trophozoites present in the intestinal segments used in this study. Since glucose is the sole carbohydrate source for Giardia trophozoites (Lindmark 1980), it is difficult to assess whether the trophozoites interfered with the transport mechanism or merely competed more successfully for the glucose.

The invasion of cells and tissues by Giardia trophozoites has been reported in humans (Morecki and Parker 1967, Brandborg 1971, Yardley et al. 1964, Saha and Ghosh 1977). In contrast to similar reports in lower animals, at least one of these reports has included transmission electron micrographs in addition to light micrographs (Morecki and Parker 1967).

The precise mechanism responsible for the pathology and symptomatology frequently seen in giardiasis remains unknown.

VII. RISK ASSESSMENT

A. Quantitative Risk Assessments

The only controlled study to determine the number of cysts required to elicit an infection in humans was performed by Rendtorff (1954). In these experiments, male volunteers were fed human-source Giardia cysts contained in gelatin capsules. Cyst dosages ranged from 1 to 1 x 10^6 cysts per capsule. Rendtorff's experiments showed that ten or fewer cysts could produce human infection (determined by observing presence of Giardia in fecal smears) (Table VII-1). Rendtorff's failure to infect five men with a dose of one cyst may have been due to feeding these volunteers inactive cysts since he did not determine cyst viability in any of these experiments. None of these volunteers exhibited symptoms that Rendtorff could associate directly with the Giardia infection.

No data on chronic exposure of humans to small numbers of Giardia cysts were located in the literature.

A number of recent studies in animals have attempted to address the dose response issue concerning giardiasis. Roberts-Thomson et al. (1976) used Swiss albino mice and G. muris cysts initially obtained from a golden hamster. Cyst morphology was used to identify the Giardia species. Mice were administered cyst dosages of 0, 1 x 10^2, 1 x 10^3 and 1 x 10^4 cysts by gavage. Twenty-two of 24 mice that received 1 x 10^2 cysts became Giardia cyst and

Table VII-1 Combined Results of Experiments Comparing the Percentage of Men Infected with the Number of *Giardia lamblia* Cysts Given

No. Cysts Given	No. Men Exposed	No. Men Infected	Percent of Men Infected
1	5	0	0
10	2	2	100
25	20	6	30
100	2	2	100
10,000	3	3	100
100,000	3	3	100
300,000	3	3	100
1,000,000	2	2	100
All doses	40	21	52.5
Control	21	0	0

Adapted from Rendtorff (1954).

trophozoite positive 5 to 14 days after inoculation. All mice that received 1 x 10^3 or more cysts became infected. Cyst viability studies in these experiments were apparently not undertaken.

Vinayak et al. (1979) used *G. lamblia* cysts isolated from humans as their cyst source for an experimental infection study in Swiss mice. Cyst dosages in this study were 0, 1 x 10^3, 1 x 10^4, 1 x 10^5 and 1.5 x 10^5. All cysts were administered by gavage. None of the control animals became infected during the 31-day experiment, but 2 of 10 mice given 1 x 10^3 cysts, 4 of 10 mice given 1 x 10^4 cysts, 7 of 10 mice given 1 x 10^5 cysts and 17 of 17 mice given 1.5 x 10^5 cysts were cyst positive for *Giardia*. Viability of the *G. lamblia* cysts was assessed using trypan blue vital stain. It must be noted that vital dye staining is not be considered an accurate assessment of *Giardia* cyst viability.

In 1982, Craft used *G. lamblia* cysts isolated from humans with symptomatic giardiasis to conduct experimental infection studies in pathogen-free Sprague-Dawley rats from the Charles River Breeding Laboratories. Cysts were administered to rats by microliter pipets in dosages of from 50 to 500 cysts, but cyst viability was not assessed. Fifty percent of the rats that were fed 50 cysts became infected while 100% of the rats fed greater than 150 cysts became infected.

Belosevic and Faubert (1983) were able to reproducibly infect CD-1 Swiss mice with as few as ten *G. muris* cysts administered by gastric intubation. The viability of the cysts in these experiments was not assessed by either vital dye staining or *in vitro* excystation. Thus, it is difficult to determine whether the cysts used in these or any of the other animal infection experiments had high levels of excystation.

The lack of data concerning the viability of *Giardia* cysts used in both animal and human dose response experiments makes determination of the minimum number of *Giardia* cysts required to establish an infection difficult, if not impossible. Theoretically, one viable cyst under optimum conditions can initiate an infection.

B. Risk Assessment Factors

Almost all people are potentially at risk from giardiasis since the infection has been reported worldwide and in virtually every state of the U.S. However, certain population groups and individuals appear to be at greater risk than the general population.

From 1970 to 1982, there were about 20,000 reported cases of waterborne giardiasis in the U.S. The actual incidence is probably much higher. The populations most at risk from waterborne giardiasis appear to be those living in New England, Pennsylvania, Colorado, California and the Pacific Northwest (Craun 1979). Craun (1979) stated that these areas traditionally depend upon surface water that is relatively free of human sewage and low in bacterial contaminants. Thus, water treatment in these areas involves primarily disinfection, not filtration. However, these states have active surveillance systems; thus, their reported cases are numerous. Other states may also have high numbers of cases; but since they have no active surveillance systems, those cases go unreported.

Since the transmission of Giardia during waterborne outbreaks appears to be directly related to the ingestion of surface water, it should be noted that surface water is the primary source of water for 18.5% of the community public water systems in the U.S. (USEPA 1983b). Calculations based on data from the EPA, Office of Drinking Water (USEPA 1983a) indicate that approximately 56 million people (Table VII-2) are potentially at risk from waterborne giardiasis via unfiltered community water systems.

Specifically, this represents the number of permanent residents serviced by community water systems (i.e., those subject to regulation under the Safe Drinking Water Act) whose drinking water: (1) is predominately from a surface water source and (2) is not subjected to treatment (e.g., filtration) that would be expected to remove Giardia cysts.

The approximately 56 million persons must be regarded as a low estimate of the total U.S. population potentially exposed to Giardia through drinking water because it does not include: (1) residents normally served by non-community water systems (e.g., national parks, campgrounds) using unfiltered surface water, (2) populations normally served by groundwater or filtered water systems which visit areas served by unfiltered surface water systems, (3) population served by filtered surface water, where filtration deficiences exist and (4) occasional consumers of untreated surface waters

Table VII-2 Estimated Number of People Potentially Exposed to Waterborne *Giardia* Infection in the United States Via Unfiltered Community Water Systems[a]

Population Category	Number of Facilities	Percentage of Total Facilities[b]	Population Served	Percentage of Total Population Served[b]
100 and under	1,247	17.4	72,000	0.1
101-500	1,883	26.3	535,000	1.0
501-1,000	970	13.5	736,000	1.3
1,001-2,500	1,232	17.2	2,072,000	3.7
2,501-3,300	265	3.7	772,000	1.4
3,301-5,000	397	5.5	1,641,000	2.9
5,001-10,000	433	6.0	3,127,000	5.6
10,001-50,000	567	7.9	12,543,000	22.5
50,001-75,000	75	1.0	4,650,000	8.3
75,000-100,000	36	0.5	3,090,000	5.5
Over 100,000	64	0.9	26,582,000	47.6
Total	7,169	100.0	55,820,000	100.0

[a] Data represent all public water systems which have at least one permanent source of water which is surface or purchased surface, and which do not filter their water (USEPA 1983a).

[b] Percentages may not add up to total due to rounding.

(e.g., backpackers, hikers, etc.). There are about 4,535 non-community water systems using unfiltered surface water, and about 1 million nonresidents who occasionally consume this water (see Table VII-3). These latter values are very approximate, and some percentage of the 1 million nonresidents are also counted among populations served by community water systems using unfiltered surface water (i.e., double counting).

Travelers to countries where water supplies are untreated or insufficiently treated are also at increased risk (Jokipii and Jokipii 1974, Weiss et al. 1977, Frost 1982, Lopez et al. 1978). There is a possible increased risk of waterborne giardiasis when *Giardia*-positive aquatic animals, especially beavers and muskrats are present in the watershed. Estimates of numbers of persons exposed to waterborne *Giardia* in these "increased risk" categories are not possible from available data.

Children in day-care centers are at increased risk from *Giardia* infections (Black et al. 1977). In these situations, however, the mechanism of transmission is probably fecal-oral and due to close association with other children and to the lower hygienic standard of the young (Black et al. 1981). Additionally, epidemiological studies suggest that these infections in children are in turn passed to other family members.

Individuals who engage in sexual activity leading to fecal-oral contact are at increased risk (Mildvan et al. 1977, Kean et al. 1979). This is especially true in areas where *Giardia* prevalence is high such as in New York City, San Francisco and London.

Table VII-3 Estimated Number of People Potentially Exposed to Waterborne *Giardia* Infection in the United States Via Unfiltered Non-Community Water Systems[a]

Population Category	Number of Facilities	Percentage of Total Facilities[b]	Population Served	Percentage of Total Population Served[b]
100 and under	3,164	69.8	137,000	12.6
101-500	1,073	23.7	249,000	22.8
501-1,000	149	3.3	116,000	10.6
1,001-2,500	89	2.0	137,000	12.5
2,501-3,300	15	0.3	45,000	4.1
3,301-5,000	22	0.5	93,000	8.5
5,001-10,000	19	0.4	152,000	13.9
10,001-50,000	3	0.1	53,000	4.9
50,001-75,000	0	0.0	0	0.0
75,000-100,000	0	0.0	0	0.0
Over 100,000	1	0.0	110,000	10.0
Total	4,535	100.0	1,092,000	100.0

[a] Data represent all public water systems which have at least one permanent source of water which is surface or purchased surface, and which do not filter their water (USEPA 1983a).

[b] Percentages may not add up to total due to rounding.

There is evidence now to suggest that Giardia infections can be spread by food handlers (Osterholm et al. 1981).

C. Reduction of Waterborne Giardiasis by Filtration

As previously stated from 1970-1982, there were at least 50 reported outbreaks of waterborne giardiasis in the U.S. with about 20,000 cases. This represents about four outbreaks and 1,500 cases annually. Between 1977-1982, there were 39 outbreaks with over 13,600 cases, representing a doubling in the annual rate to eight outbreaks and 2,700 cases. This increase may be due to greater recognition and reporting of giardiasis outbreaks in recent years. Table VII-4 identifies the water treatment deficiency associated with many of them (Craun 1984). Most outbreaks (67%) and most disease (52%) resulted from contamination of untreated surface water or surface water with disinfection as the only treatment.

Using the above information, one can calculate the anticipated decrease in outbreaks if all systems using surface water included filtration. Assuming an annual rate of eight outbreaks and 2,700 cases, then about 8 x 67% = 5.3 outbreaks and 2,700 x 52% = 1,400 cases could be prevented annually.

The major deficiency in these calculations is that outbreaks of waterborne giardiasis are probably still grossly under-reported (see Chapter III). The waterborne disease surveillance system is generally a passive one, and four States which have active surveillance have reported more than half of all outbreaks of giardiasis (Craun 1984). In one state which

Table VII-4 Water System Deficiencies Responsible for Waterborne Outbreaks of Giardiasis in the United States, 1965-1980[a]

Deficiency	Outbreaks	Cases
Surface water, chlorination[b]	18	10,045
Surface water untreated	10	312
Ineffective filtration	5	6,981
Groundwater, untreated	2	19
Groundwater, chlorination only	1	123
Cross-connection	3	2,209
Contaminated cistern	1	5
Unknown	1	19
Interrupted disinfection of spring	1	15
Total	42	19,728

[a] Data for 1981 not readily available for this analysis.

[b] Includes one outbreak where filtration facilities were available but used intermittently.

Adapted from Craun (1984).

switched from a passive to an active system, annual reported outbreaks increased by a factor of 3.5 (USPHS 1982c). If the number of giardiasis outbreaks and cases is 3.5 times higher than reported, and if one assumes that the deficiency type proportions suggested in Table VII-4 are similar with additional data, then filtration could prevent 3.5 x 5.3 outbreaks = 18.5 outbreaks and 3.5 x 1,400 cases = 4,900 cases.

Another deficiency in the calculations is that it is assumed that all filters installed on either untreated or disinfection-only-treated surface waters will be effective for cyst removal. A less significant deficiency is that the outbreak values used in the above calculations include private systems (between 1978-1982 only two outbreaks with 22 cases were reported for private systems).

D. Summary and Quantification of Toxicological Effect

Because of the prevalence and distribution of *Giardia*, virtually all people, regardless of age, sex or socioeconomic background, are considered to be at risk. About 56 million people in the U.S. are estimated to be at potential risk from giardiasis via community drinking water systems (i.e., those using surface water that has not been filtered to remove *Giardia* cysts).

Individuals and populations considered to be at greatest risk are (1) those who drink surface water treated by disinfection and not by filtration, (2) those who drink untreated water in recreational areas, (3) children in day-care facilities and their families, (4) individuals who engage in sexual activities leading to fecal-oral contact and (5) foreign travelers.

Based on the prisoner study by Rendtorff (1954), ten cysts can cause an infection in healthy humans. The cyst challenge level, however, may have been lower in this study, since cyst viability was not determined. In unpublished USEPA data, the ID(50)'s for mice challenged with *Giardia* *muris* cysts in four experiments were 1.4, 2.6, 3.8 and 17.6 cysts. If ID(50)'s for mice are this low, then presumably one cyst infects a high percentage of these mice. Based on this information, the safety factor on the human data should be greater than 10. Thus, the recommended acceptable daily intake =

$$\frac{\text{minimum effect level}}{\text{safety factor}} = \frac{10 \text{ cysts}}{>10} = <1 \text{ cyst}$$

Therefore, there is no supportable scientific basis for an acceptable daily intake other than zero. The National Academy of Sciences and USEPA's Cancer Assessment Group have not addressed risk levels for *Giardia*.

VIII. TREATMENT AND ANALYSIS OF *GIARDIA*

A. Treatment in Water

Being prepared by different authors under another contract.

B. Analysis in Water

1. Techniques of Sampling Water for *Giardia* Cysts

Methods of *in vitro* cultivation of *Giardia* trophozoites (Meyer and Pope 1965, Meyer 1970, Meyer 1976, Danciger and Meyer 1971) and *in vitro* excystation of *Giardia* cysts (Bingham and Meyer 1979, Bingham et al. 1979, Rice and Schaefer 1981) have been developed. Despite this, no standard cultivation method exists for the detection of *Giardia* in water (Jakubowski and Ericksen 1979). This is undoubtedly due to the difficulty of establishing *in vitro* cultures (Meyer 1979). Jakubowski and Ericksen (1979) concluded that animal feeding experiments and direct microscopic examination of water were alternatives to cultivation, but that these methods also have caveats. For example, animal feeding experiments require special handling and isolation facilities for specific-pathogen-free animals (Jakubowski and Ericksen 1979, Grant and Woo 1978b, Craft 1982, Sebesteny 1979) and are time-consuming and expensive (Jakubowski and Ericksen 1979).

Direct microscopic examination of water samples for *Giardia* cysts is also very labor-intensive and requires a skill in cyst identification not readily available in most water supply laboratories (Jakubowski and Ericksen 1979). Jakubowski and Ericksen (1979) further reported the results of a CDC proficiency testing program which suggested that from 20 to 35% of public and private laboratories could not correctly identify the cysts of intestinal protozoans.

With this in mind, Jakubowski and Ericksen (1979) concluded that microscopic detection of cysts from water samples was the only practical method presently available for *Giardia* cyst detection. The microscopic examination of water samples requires the sampling of large volumes of water (Jakubowski et al. 1978). The only feasible way of sampling large volumes of water is by concentrating the samples by filtration.

Moore et al. (1969) took ten 2-L water samples from various locations throughout the city of Aspen, CO, in their investigation of the 1965-66 outbreak of giardiasis. A 1-L sewage sample was also taken from each of three main sewage trunk lines. These samples were filtered through cheesecloth and then through a 0.45-μm membrane filter. The sediment was collected in water, centrifuged and preserved in formalin for microscopic examination. *Giardia* cysts were detected in the sewage but no cysts were detected in the water samples.

In another outbreak of giardiasis in campers, 22 L of stream water were passed through a membrane filter and the sediment was examined microscopically for *Giardia* cysts (Barbour et al. 1976). No *Giardia* cysts were found.

Shaw et al. (1977) sampled much larger volumes of water in an investigation of the Rome, NY, outbreak. These investigators used a swimming pool filter with sand as the filter medium to filter 1.1×10^6 L of raw water. They pumped 76 L/minute daily for 10 days and collected the filter backwash each day. The filter backwash was placed in 55-gallon drums and coagulated with alum. The sediment from the coagulation containers was removed and used for animal feeding experiments and for microscopic examination at the CDC. As a result of this method, one cyst was detected microscopically and two of ten challenged dogs were infected using the sediment collected.

In the Berlin, NH, outbreak of 1977, Lippy et al. (1978) devised a sampling method that also involved filtration. They removed the bottom of a plastic bottle and placed gauze filter pads at the bottle neck. Cysts were reportedly identified from the material trapped in the gauze.

A method for the collection of Giardia cysts from large volumes of water was developed by the Environmental Protection Agency (EPA) (Jakubowski et al. 1978). This sampling method is an adaptation of a large-volume method used for sampling water for viruses. Jakubowski and co-workers (1978) used an Orlon fiber filter with 10-μm porosity for filtering water in the Montgomery Center, VT; Camas, WA; Berlin, NH; and Granby, CO, outbreaks. Cysts were detected in all of these investigations.

Jakubowski and Ericksen (1979) suggested an improvement in the Orlon filter would be to decrease the porosity of the filter to 7 μm since the retention of cysts should be greater than 95% efficient at this pore size.

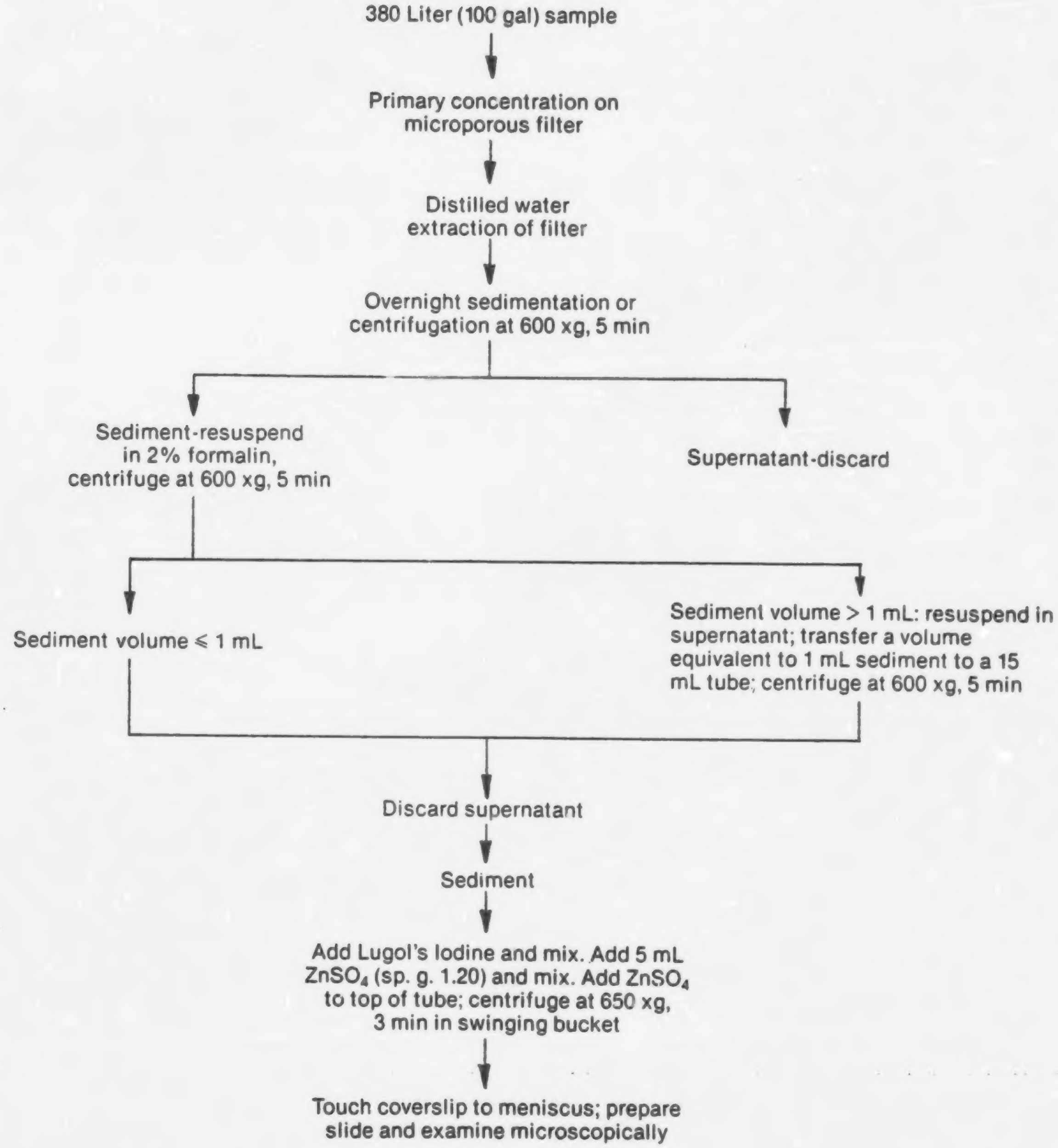
The method presented by Jakubowski (in press) is currently the method recommended for sampling water for Giardia cysts. A schematic flow chart of this procedure is presented in Figure VIII-1.

2. Determination of Cyst Viability

Three methods for the determination of cyst viability have been discussed in previous sections of this paper, including eosin exclusion (vital dye) test, in vitro excystation and animal infectivity (Bingham et al. 1979, Grant and Woo 1978b, Rice and Schaefer 1981). Of these three methods only the latter two have been widely accepted as reliable methods for determing Giardia cyst viability. No study has been conducted to correlate in vitro excystation with animal infectivity. Thus, APHA-AWWA-WPCF (1981) recommends the use of animal feeding experiments similar to those described by Shaw et al. (1977) for the determination of Giardia cyst infectivity. It is recommended, however, that these experiments be conducted only by laboratories equipped with necessary isolation and handling facilities.

C. Analysis in the Host

The analysis of Giardia infection in the host population might serve as a method for determining the contamination potential in a community and thus serve to trigger improved water treatment. A complete review of the standard methods for the clinical detection of Giardia in humans has been written by Wolfe (in press).


380 Liter (100 gal) sample
Primary concentration on microporous filter
Distilled water extraction of filter
Overnight sedimentation or centrifugation at 600 xg, 5 min
Sediment-resuspend in 2% formalin, centrifuge at 600 xg, 5 min
Supernatant-discard
Sediment volume ≤ 1 mL
Sediment volume > 1 mL: resuspend in supernatant; transfer a volume equivalent to 1 mL sediment to a 15 mL tube; centrifuge at 600 xg, 5 min
Discard supernatant
Sediment
Add Lugol's Iodine and mix. Add 5 mL ZnSO4 (sp. g. 1.20) and mix. Add ZnSO4 to top of tube; centrifuge at 650 xg, 3 min in swinging bucket
Touch coverslip to meniscus; prepare slide and examine microscopically

Adapted from Jakubowski (In Press)

FIGURE VIII-1 PROCESSING SCHEME FOR GIARDA WATER FILTERS

The initial procedure employed to diagnose giardiasis is the demonstration of the causative organisms, either in the cyst or the trophozoite form, in the host's feces. The microscopic examination of three fecal samples obtained at 48-hour intervals will successfully yield organisms in 90% or more of the cases of giardiasis (Meyer and Jarroll 1980). In a heavy infection with large numbers of cysts in the stool, direct microscopic examination of a fecal smear in saline or Lugol's iodine may be sufficient. In light infections or when direct smears are negative, formolether concentration procedures or zinc sulfate flotation should be performed. Wolfe (in press) points out that purgatives do not appear to aid in the diagnosis and that no routine stool culture methods are presently available. Furthermore, many antibiotics, antacids, kaolin products, paregorics, most enema preparations and oily laxatives may cause a masking or disappearance of the parasites from the feces temporarily. The examination of stool or intestinal contents for Giardia should be delayed from five to ten days after the use of such products.

In humans whose undiagnosed gastrointestinal illness is suggestive of giardiasis, but whose feces are consistently free of the etiological agent, diagnosis may require obtaining and identifying motile trophozoites in specimens from the upper small intestines. Such specimens of intestinal fluids may be obtained by a duodenal tube or with the Enterotest (HEDECO Company, Mountain View, CA 94043). The Enterotest consists of a weighted gelatin capsule containing a nylon thread (both adult and children sizes are available). With the free end of the thread taped to the patient's face, the capsule is swallowed and as the gelatin dissolves the string unwinds. Usually within four hours the thread has extended to its full length and may be removed. The string which is saturated with mucus is then removed and examined microscopically for Giardia trophozoites. Wolfe (in press) discusses conflicting reports on the success of examining duodenal fluids. His personal observations suggest that after a series of negative stools, it is difficult to confirm an infection by examining intestinal fluids. His recommendation is to supplement stool examinations with intestinal fluid analysis rather than to substitute the fluid analysis for stool examinations.

Small intestinal biopsies may occasionally be used when Giardia infection cannot be confirmed after multiple stool examinations or by the examination of intestinal fluids.

Gastrointestinal radiology using barium may show particular diagnostic features of giardiasis in 20% of the patients infected (Wolfe in press). Such features include thickening of the mucosal folds in an irregular fashion, spasticity of the gut, particularly in the duodenum and proximal jejunum, and fragmentation of the barium column due to increased intestinal secretions. However, the use of this procedure should follow those discussed above since the barium will make it impossible to detect Giardia for a week or longer.

It should be noted that the Enterotest, duodenal aspiration and biopsies, as well as the radiological techniques would not lend themselves to routine screening of humans or animals for Giardia infection.

There are presently no clinically accepted serological methods for detecting giardiasis. However, several recent reports of experimental serological procedures have previously been discussed in Section V.C.3., Diagnostic Tests. Additionally, Riggs et al. (1983) reported the development of an immunofluorescence technique for the detection of G. lamblia cysts in feces. In this procedure, G. lamblia cysts were purified from human feces and high-titer immune sera were raised in guinea pigs. These sera were labeled with fluorescein isothiocyanate and used to detect cysts by epifluorescence microscopy. Cross reactivity of the immune sera with Chilomastix mesnili was as intense as reactivity with G. lamblia, but no cross reactions were observed for Iodamoeba butschlii, Dientamoeba fragilis, Entamoeba histolytica, Entamoeba coli or Endolimax nana. These authors were able to distinguish G. lamblia from C. mesnili morphologically even when stained. This technique, although presently used for the detection of G. lamblia cysts in feces, could potentially be applied to the detection of Giardia cysts in concentrated water samples.

D. Summary

A method for analyzing water for Giardia cysts is available. The method is at present considered tentative and experimental. Basically it involves filtering 380 L through a microporous filter. The sediment collected from the filter is concentrated and examined microscopically. If cysts are found, aliquots of the sediment are fed to specific-pathogen-free animals. Cyst viability and infectivity are determined by these animal feeding experiments and it is recommended that the feeding experiments be conducted only by laboratories specially equipped for this purpose. The analysis of Giardia infection in the host population can be performed by (1) direct microscropic examination of fecal smears for cysts, (2) identification of motile trophozoites in specimens from the upper intestines, (3) intestinal biopsies or (4) gastrointestinal radiology using barium. However, none of the latter methods would lend themselves to routine screening for giardiasis.

The potential exists for the development of an immunofluorescence test to detect Giardia cysts in concentrated water samples.

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